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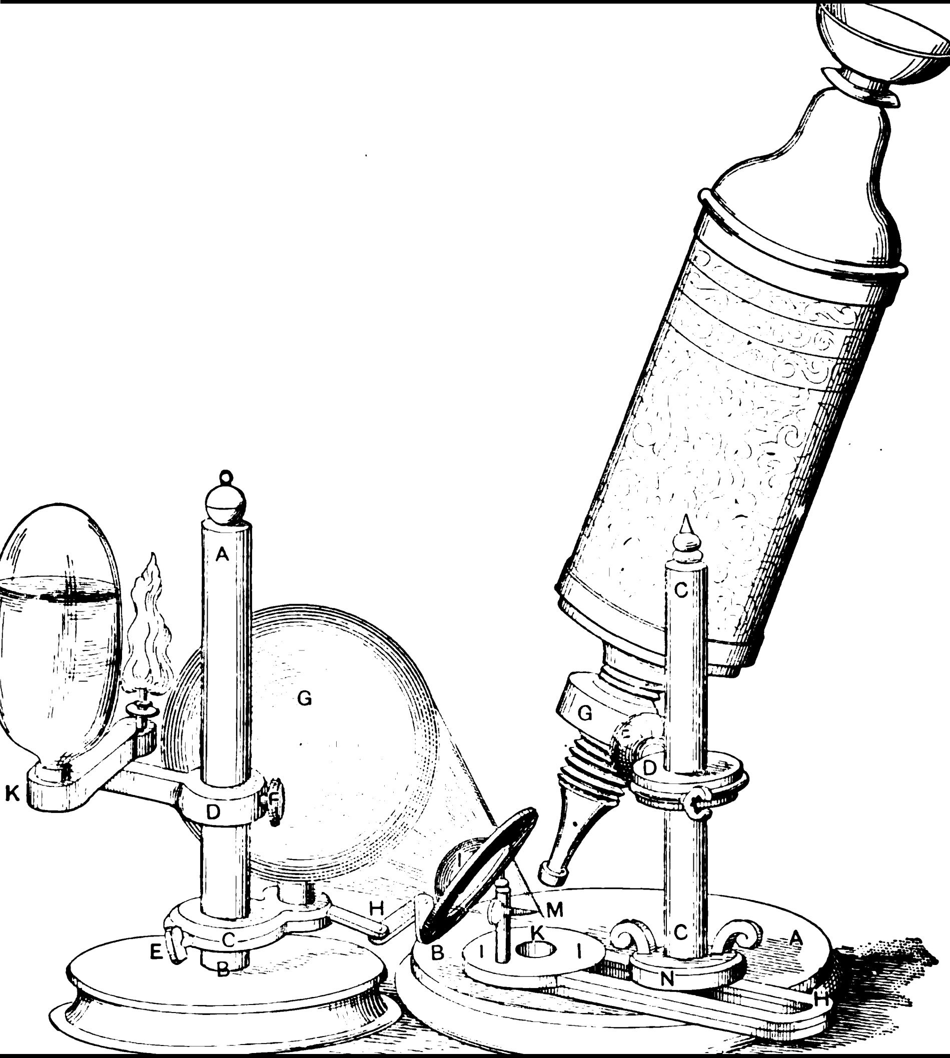
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Elements of applied microscopy

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OF

APPLIED MICROSCOPY.

A TEXT-BOOK FOR BEGINNERS.

BY

CHARLES-EDWARD AMORY WINSLOW,
*Instructor in Industrial Microscopy and Sanitary Biology in the
Massachusetts Institute of Technology.*

FIRST EDITION.
FIRST THOUSAND.

NEW YORK:
JOHN WILEY & SONS.
LONDON: CHAPMAN & HALL, LIMITED.
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DEDICATED
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To His Mother.

INTRODUCTION.

THIS little book is intended for the teacher, and the beginner with the microscope, not for the specialist. It contains very few original data and treats no single subject with completeness.

Almost all the branches of technical microscopy have been already made the basis for monographs with which the present volume can in no way compete. On the other hand, there are many entertaining popular books on the microscope which treat it as a mere adjunct to the study of natural history. Neither type of work was suited for the use of a class in Industrial Microscopy which is offered to second-year Chemists and Biologists at the Massachusetts Institute of Technology. The object of this course is twofold—first, to give facility in the manipulation of the microscope; and second, to furnish an acquaintance with the scope of its practical application. As a text-book there was needed a brief and elementary treatise which should take up the fundamentals of the science and art of microscopy itself and make a rapid but wide survey of the principal fields in which the microscope has been applied to practical affairs. No such elementary but comprehensive work exists in English among the numerous able treatises upon special branches of the subject. The present volume is therefore the outgrowth of a pedagogic need. The book is necessarily incomplete from the standpoint of the expert in any of the branches which it treats, but if it conveys to the student's mind such an idea of the possible applications of the microscope in varied fields as shall stimulate him eventually to the further exploration of some one of them, it will have served its purpose.

In the preparation of this volume, the authorities quoted in connection with the various chapters have been freely drawn upon. The author further desires to express his grateful obligation, to Dr. P. G. Stiles for the preparation of original drawings; and for advice and assistance in regard to various portions of the manuscript to Professor W. T. Sedgwick, Professor Mary A. Willcox, Professor H. M. Goodwin, Professor F. J. Moore, Professor C. H. Warren, Dr. C. C. Simmons, Mr. A. E. Leach, Mr. A. G. Woodman, Dr. E. L. Walker, and Miss A. F. Rogers.

Acknowledgments are due to the authors and publishers of the following books for figures which have either been copied directly or redrawn:

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ELEMENTS OF APPLIED MICROSCOPY.

CHAPTER I.

FUNCTION AND PARTS OF THE MICROSCOPE.

1. **Normal Vision.**—In the ordinary act of vision at least two distinct processes may be distinguished, the formation upon the retina (*K*, Fig. 1) of a minute image of the object seen, and the transformation, by the optic nerves and their end organs, of the energy of the light-waves thus thrown upon the retinal surface into that form of nervous activity which we call sensation. The first process follows the simple laws of Optics, while the second is controlled by the far more complex chemical and physical conditions which furnish the subject-matter for the science of Physiology. The sensation experienced is directly related to the image lying upon the retina; that is, to an area of special illumination whose parts correspond to those of some object outside, from which the illumination is derived. The mind has learned, by experience, to interpret a certain image upon the retina

as corresponding to a definite object perceived by the other senses. The image is in all cases inverted, but in infancy it is found that images upside down correspond to objects which are really right side up. Experience teaches, also, that an image of a certain size produced by an object at a given distance always implies a definite magnitude; and we are therefore able to judge something about the actual size of an object from the size of its image and our knowledge of its remoteness.

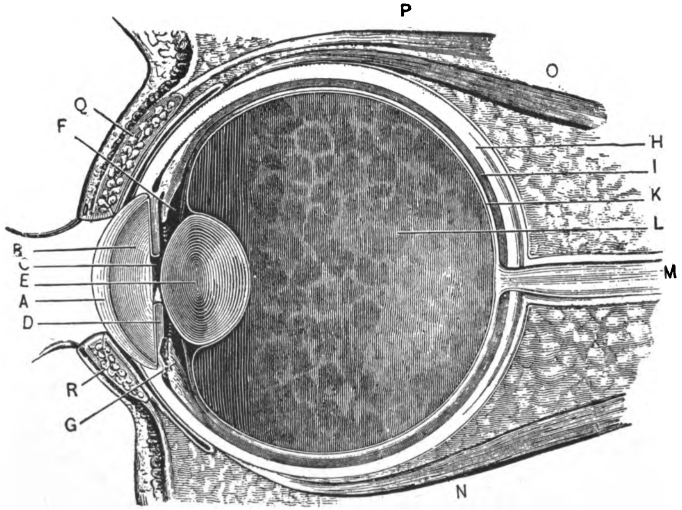


Fig. 1.—Section through the Human Eye.
(After Everett-Deschanel.)

A. Cornea.
B. Aqueous humor.
C. Pupil.
D. Iris.
E. Lens.
F. Suspensory ligament.
G. Accommodation muscle.
H. Sclerotic coat.
I. Choroid coat.
K. Retina.
L. Vitreous humor.
M. Optic nerve.
N. Inferior rectus muscle.
O. Superior " "
P. Levator palpebræ muscle.
Q. *R.* } Meibomian glands.

2. Function of the Microscope.—In order that a visual image may be produced, a certain definite area of the retina must be stimulated, and objects whose images are smaller than this minimum will ordinarily be invisible. Normal vision is therefore limited to fairly large objects near at hand. If, however, the rays proceeding from more remote, or smaller, objects can be collected by a lens and bent so as to produce a larger image, the objects may be seen. This end is attained by the telescope and the microscope,—the former producing enlarged images of large and remote objects, the latter producing enlarged images of near and minute objects.

3. Laws of Refraction.—The formation of such images by the microscope is dependent upon the fact that rays of light in passing from any medium to one of different density experience a change in direction, unless they impinge at right angles to the surface of contact.

The deflection thus produced has been compared to the alteration in the course of a column of troops on passing from a smooth parade-ground into a ploughed field. If, in Fig. 2, aba_1b_1 represents a body of troops marching in the direction indicated by the arrow, it is apparent that the men on the right of the line will reach the ploughed field $b_1a_1b_2a_2$ first and will be somewhat retarded. The left of the line will gain upon the right and the column as a whole will execute a partial right face. In passing out of the field on the other side, the right of the line will reach the smooth ground first and gain as much as it lost before, the final result being that the column proceeds in a direction parallel to that which it originally pursued.

It will be noted that in passing into the region offering greater resistance the marching column takes up a direction more nearly at right angles to the boundary of that region; in other words, it is deflected toward a line drawn normally, or at right angles, to that boundary (ll_1). So

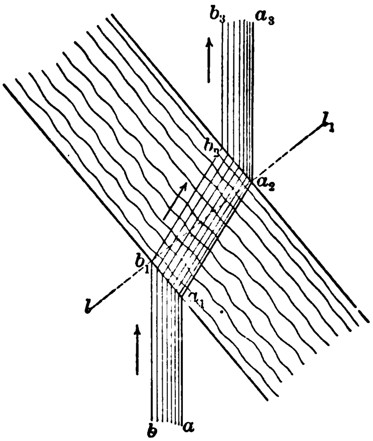


FIG. 2.—ILLUSTRATION OF REFRACTION. (After Hager-Mez.)

when a ray of light passes from a less dense to a more dense medium it is always refracted toward the normal, while in passing from a more dense to a less dense medium it is bent away from the normal.

4. **Refraction in the Convex Lens.**—A ray of light passing through a piece of glass with parallel sides will simply be shifted laterally, as in our illustration of the marching column. In passing through a prism, however, a resultant bending ensues as shown in Fig. 3.

Ray *AB*, bent toward the normal at *B*, turns to the right and, bent away from the normal to the surface, at *C*, turns to the right still further. If two such prisms are placed base to base as in the figure, rays striking them, as do the lines *AB* and *AB′*, will be bent in opposite directions so that they will meet at the point *D*. The amount of bending varies with the angle at the apex of the prism. As shown, for example, in Fig. 4, the ray *a′b′c′d′* passing through a prism of wide angle will suffer greater deflection than the ray *abcd* which meets surfaces to which it is more nearly normal.

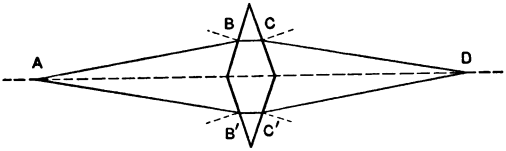


Fig. 3.—Refraction by Prisms.

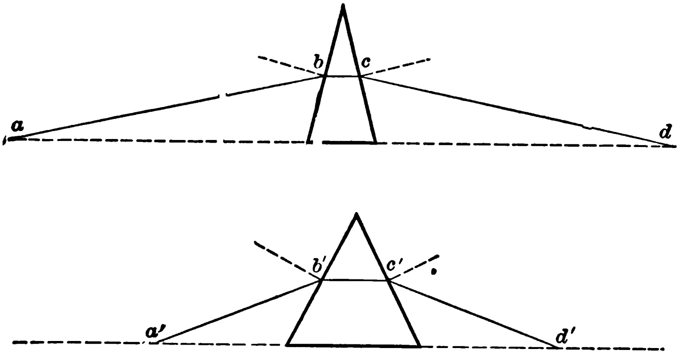


Fig. 4.—Refraction in Prisms of Differfnt Angles.

A biconvex lens works roughly, as if it were made up of an infinite number of prisms arranged about a central axis. Rays parallel to the principal axis of such a lens (the line joining the centers of curvature of its two surfaces,—will be bent from all directions toward that axis, and the rays nearest the outer edge or periphery will be most bent, so that all will meet at one point, known as the Principal Focus or burning-point of the lens (*o*, Fig. 5). The greater the curvature of the lens the nearer to its optical center this principal focus will lie.

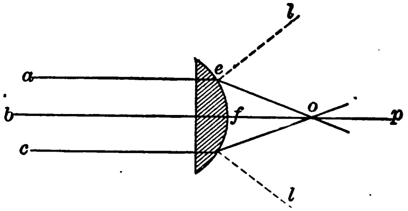


FIG. 5.—PLANO-CONVEX LENS AND ITS PRINCIPAL FOCUS. (After Hager-Mez.)

5. Formation of Images by the Convex Lens.—As the principal focus of a biconvex lens is defined as the point at which rays parallel to the principal axis meet, it is obvious that rays proceeding from a radiant point placed at the principal focus will be sent off on the other side of the lens as parallel rays, and will meet to form an image only at an infinite distance (*B*, Fig. 6). Rays from a point between the lens and its principal focus, being still more divergent, will not even be made parallel, but will still continue to diverge after passing through the lens, though of course less so than before (*A*, Fig. 6). No real image can therefore be formed by an object lying inside the principal focus. Rays from a point outside the principal focus have an initial divergence so small as to be entirely overcome by the lens. Such rays (*C*, Fig. 6) will actually converge after passage through the lens and will meet at a definite point to form a real image.

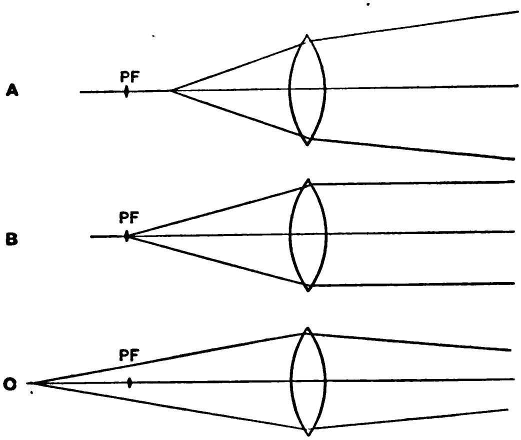


Fig. 6.—Course of Rays according to the Relation of a Luminous Point to the Principal Focus.

6. Construction of Real and Virtual Images.—The construction of the image formed by any object is very simple, if only its relation to the principal focus of the lens be determined. The course of two rays from any point is known with certainty. The ray parallel to the principal axis will be so bent as to pass through the principal focus on the opposite side of the lens. The ray passing through the optical center of the lens will not be deflected at all, because it will cut surfaces which are parallel to each other. Where these two rays meet the image of the point must be formed. Thus in Fig. 7 is shown a biconvex lens the surfaces of which have an equal curvature; its principal foci, therefore, lie at equal distances from its center. The object *ab* lies outside the principal focus *F*. The image of the point *a* is

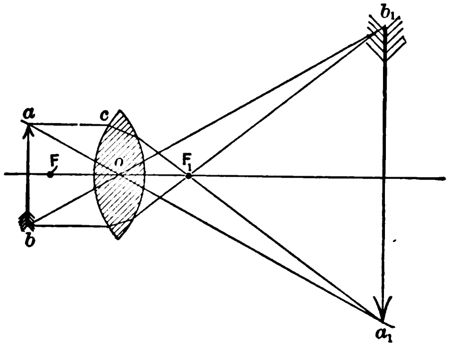


Fig. 7.—Formation of Image by Object outside the Principal Focus. (After Hager-Mez.)

determined by the straight line aa_1 and the broken line aca_1. The image of the point *b* is similarly fixed, and between the two will be formed the enlarged image *a′b′*. This will be a real image—one, that is, which could be caught upon a screen held in the right plane,—and it will be inverted. These are the characteristics of all images formed by objects lying outside the principal focus. Fig. 8 illustrates the other case, in which the object lies inside the principal focus. Here it is evident that the rays from the point *a* will not meet at all on the opposite side of the lens and therefore no real image can be formed. They will appear to the eye to come from a point more remote than their true position; and the exact location of this point may be found by prolonging the lines from *o* and *F* backward until they meet. This image will not be inverted, but erect; and as it cannot be caught upon a screen it is called a virtual image.

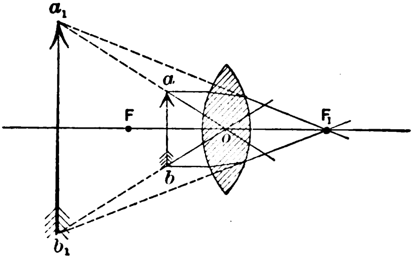


FIG. 8.—FORMATION OF IMAGE BY OBJECT INSIDE THE PRINCIPAL FOCUS. (After Hager-Mez.)

7. **Development of the Simple Microscope.**—The effect of globes of crystal in concentrating the sun's rays on a single point was known in very early times. Aristophanes, Pliny the Elder, and other Greek and Roman authors mention the use of such primitive burning-glasses. Seneca states that "letters though small and indistinct are seen enlarged and more distinct through a globe of glass filled with water." None of the ancients appear, however, to have thought of any practical application of this phenomenon as an aid to vision; and medical writers up to the thirteenth century of the Christian era speak of short-sightedness as an incurable infirmity.

In microscopy, as in so many other branches of knowledge, the first distinct advances were made in Arabia. Alhazen, the great Arabian physician of the eleventh century, distinctly describes the use of lenses for producing enlarged images, in a publication supposed to date from about 1052. Two centuries after, Roger Bacon, the Franciscan monk, and noted alchemist, of Oxford, noted the same effect. A little later, near the end of the thirteenth century, lenses were first applied to the mitigation of defects of vision; and to Salvino d'Armato degli Armati, a Florentine, the invention of spectacles is ascribed. The use of lenses as microscopes for the examination of objects too minute to be studied with the unaided eye became general about the end of the sixteenth century. From the year 1600 such observations were numerous, and in 1637 the first diagram of a microscope now extant was published by Descartes. About 1665 small glass globules began to be used instead of convex lenses for the simple microscope. They were set in metal plates, on the side of which opposite to the observer the object to be examined was mounted on some sort of movable arm. With these instruments a high magnification was obtainable; and it was with such simple microscopes (Fig. 9) that the pioneer microscopists of the seventeenth century, Kircher in Italy and Leeuwenhoek in Holland, founded the science of Micro-Biology.

8. The Compound Microscope.—Meanwhile a step had been taken, which, though not particularly fruitful at the time, was to become later of great significance. This was the invention of the compound microscope, commonly attributed to the Dutch spectacle-makers, Hans and Zacharias Janssen, and supposed to date from about 1590. It is certain that a compound microscope was independently invented by Galileo in 1610, and that Cornelius Drebbel in Holland was credited with the introduction of the instrument about 1621. These early instruments and their illuminating apparatus were cumbrous and unwieldy in the extreme (see Fig. 10).

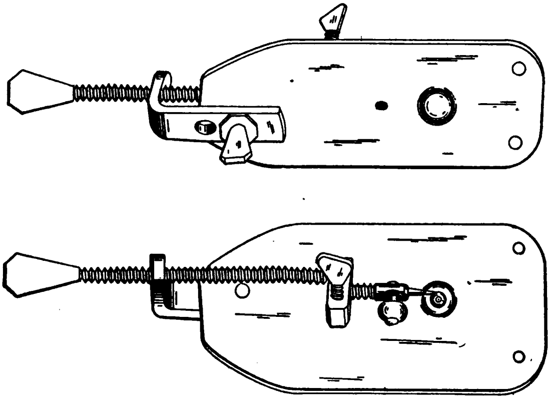


FIG. 9.—LEEUWENHOEK'S MICROSCOPE (CIRCA 1700). (After Carpenter-Dallinger.)

The compound microscope is characterized by the fact that it contains two or more lenses or systems of lenses, one of which forms an image of the object, while the other forms a second image of the first image. The course of the rays in such an instrument is shown in Fig. 11. The object AB lies outside the principal focus of the lens system marked Objective, and its real inverted image is formed at A^1B^1. This image is produced at a point inside the principal focus of the Ocular or Eyepiece; and the eyepiece forms an erect and virtual image, A^3B^3, of the real inverted image A^1B^1.

9. Defects of the Compound Microscopes.—The compound microscope was at first of little practical importance, on account of the complications introduced by spherical and chromatic aberration. The former is due to the fact that the rays which pass through the outer

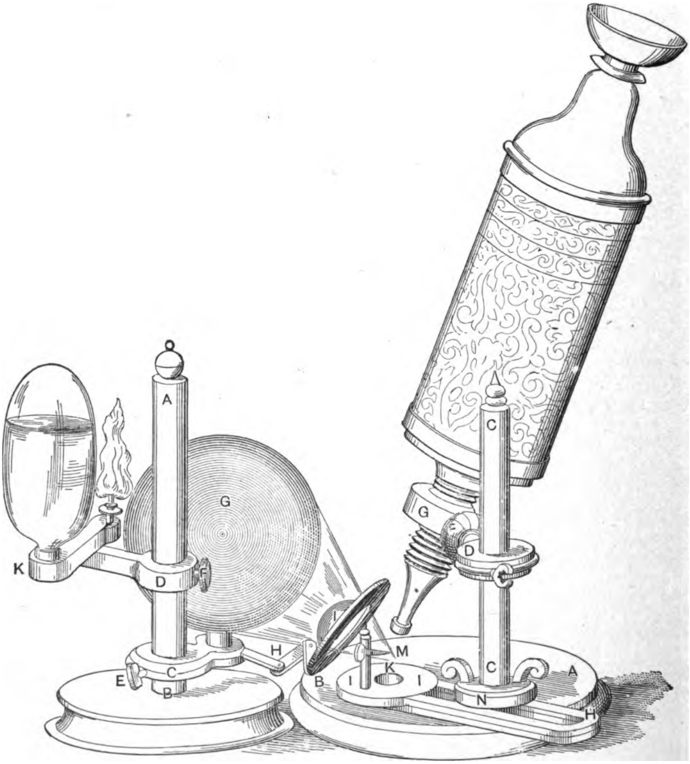


FIG. 10.—HOOKE'S COMPOUND MICROSCOPE (1665). (After Carpenter-Dallinger.)

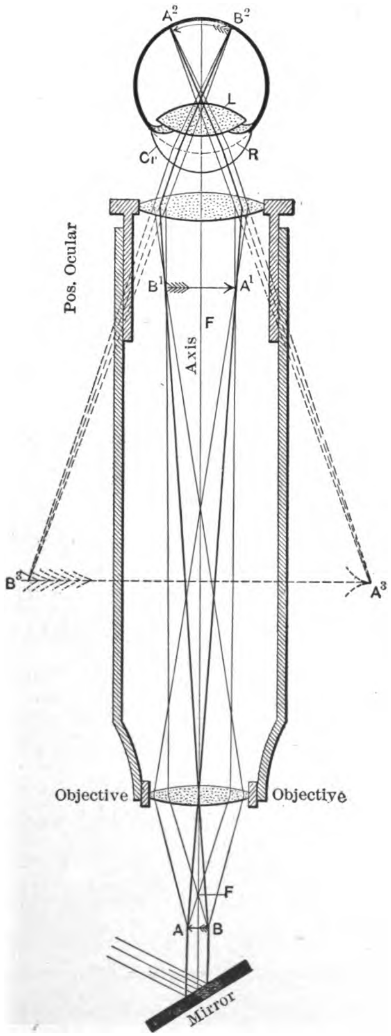

A²
B²
L
Cr
R
Pos. Ocular
B¹
A¹
F
Axis
B³
A³
Objective
Objective
F
A
B
Mirror

FIG. 11.—COURSE OF RAYS IN THE COMPOUND MICROSCOPE
(After Gage.)

part or periphery of a biconcave lens are brought to a focus somewhat closer to the lens than those which lie nearer the optic axis. This deflection and the consequent blurring of the image increase with every increase in the curvature of the lens. A still more serious defect lies in the fact that the rays of light of different colors are differently affected by the ordinary lens, those of shorter wave-length at the violet end of the spectrum, (*v*), coming to a focus first, as indicated in Fig. 12. The result is that rings of spectral colors are produced bordering the objects examined; not serious in the simple magnifiers, this effect is greatly exaggerated by passing through the long distance between the objective and the eyepiece of the compound microscope. For two centuries after the construction of these instruments they were so subject to spherical and chromatic aberration as to have little practical value, and were on the whole inferior to the best simple microscopes.

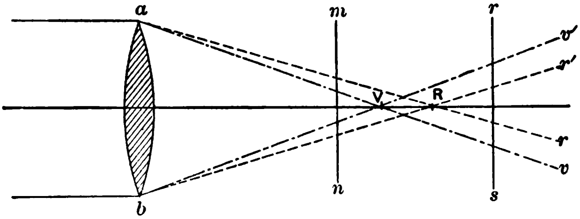


FIG. 12.—CHROMATIC ABERRATION. (After Hager-Mez.)

10. **The Achromatic Objective.**—The third decade of the nineteenth century saw these difficulties overcome. It had long been recognized that in different sorts of glass the refractive or bending power and the dispersive power

(in virtue of which white light is split up into its components) varied independently of each other. Thus flint glass has only .1 to .2 more refractive power than crown glass with more than twice its dispersive power. If a biconvex lens of crown glass, (*C*), be combined with a plano-concave lens of flint glass, (*F*), arranged in the opposite sense, as shown in Fig. 13, it is possible so to adjust their opposite curvatures that the flint glass shall compensate for all the dispersion caused by the crown glass and neutralize only half its refraction. This is the principle of the achromatic objective; and through the efforts of Selligues and Chevalier in France, Fraunhofer in Germany, Amici in Italy, and Goring, Tulley, and Lister in England, it gradually attained its practical development between 1820 and 1830. With this improvement the compound microscope acquired new importance; and it very shortly developed into one of the most important instruments at the disposal of modern science.

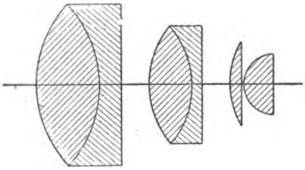


FIG. 13.—THE ACHROMATIC OBJECTIVE. (After Hager-Mez.)

11. The Immersion Objective.—Lenses made of combined crown and flint glass in such fashion as to be achromatic will also show a decreased spherical aberration, and this latter defect may be further reduced to a minimum by adjusting the radii of curvature of the opposite surfaces of the lenses according to certain known relations. Such lenses are designated as aplanatic.

There still remained one serious limitation to the increase of the power of magnification of the compound microscope. In all its various refractions there is a loss of light, and with the small objective lenses of great curvature, necessary for high magnification, it is difficult to get a sufficient illumination for clear vision. Furthermore, it has been shown by Abbé and others that the rays which extend from a point toward the periphery of a lens are of prime importance in the detection of the finer structure of objects. If the outer zones of rays are lost, no image of very minute details can be formed; and this is just what occurs in the ordinary compound microscope when there is air between the objective and the specimen to be examined, as is shown in the right half of Fig. 14. If, on the other hand, some substance like cedar-oil, which has the same refractive index as glass, be placed between the lens and the cover-slip which covers the specimen, the rays will

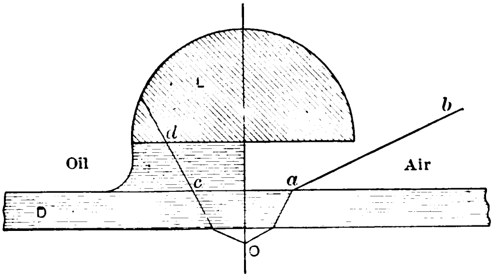


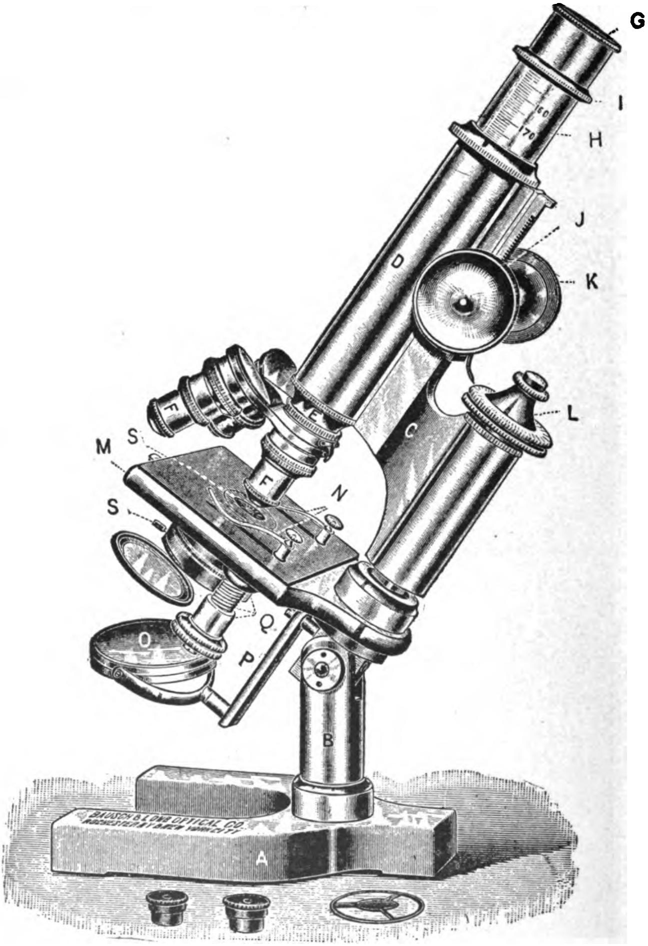
FIG. 14.—EFFECT OF HOMOGENEOUS IMMERSION. (After Hager-Mez.)

take the direction indicated on the left-hand side, more peripheral rays will enter the lens, and more detailed images will be produced. Such an immersion objective was first suggested by Amici in 1850, and next to the achromatic objective this may be considered the greatest single step in the improvement of the compound microscope.

The degree to which a lens admits the peripheral rays of light is designated by the term angular aperture, which signifies the angle contained between the most divergent rays passing through the objective from the axial part of an object (a point situated on the principal axis of the lens). Obviously the angle will increase with the convexity of the lens and its consequent short focal distance; with the same lens, the angle will be greater when some homogeneous immersion substance is used. Taking this factor into account, the power of an objective to collect and utilize divergent light-rays is called its Numerical Aperture; this quantity is equal to the index of refraction of the medium in front of the lens multiplied by the sine of half the angle of aperture.

12. The Mechanical Parts of the Microscope.—The microscope consists in its essentials of the two systems of lenses of which we have spoken. For steadiness these optical parts must be mounted upon a rigid stand; and in addition apparatus is needed for throwing light upon the object to be examined and for focusing, or so adjusting the relation between the object and the lenses that a clear image may be produced.

A microscope of the ordinary American pattern, illustrated in Fig. 15, has a heavy horseshoe-shaped base


G
I
H
160
170
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B
BAUSCH & LOMB OPTICAL CO.
ROCHESTER N.Y. & NEW YORK CITY
A

FIG. 15.—THE MICROSCOPE.

A. Base.
B. Pillar.
C. Arm.
D. Tube.
E. Collar.
F. Objectives.
G. Ocular
H. Draw-tube scale.
I. Draw-tube.

J. } Coarse adjustment.
K. }
L. Fine adjustment.
M. Stage.
N. Spring clips.
O. Mirror.
P. Mirror bar.
Q. Substage.
S. Diaphragm.

and a vertical pillar rising from it, which may or may not be jointed. Attached to the pillar is the stage, a perforated plate upon which the object may be placed; below this is the lighting mechanism, and above is the arm carrying the optical parts.

The lighting apparatus includes first a mirror, usually having one plane and one concave surface and so mounted on a jointed bar as to set at any angle. For high powers it is customary to concentrate the light from the mirror by means of an Abbé condenser, a large lens placed below the stage.

The stage itself in the simpler microscopes is merely a flat plate with an opening in the center, and with clamps for holding the slide upon which the object is mounted. For delicate work it is convenient to use a microscope equipped with a mechanical stage which can be moved forward and backward and from side to side by a micrometer screw. Such an arrangement makes it easier to explore the whole of the specimen to be examined, and enables one to find any particular portion of it at pleasure. Under the stage, whether it be of the simple or the mechanical type, there should be some sort of diaphragm for regulating the direction from which light will reach the object, and cutting off the peripheral rays coming from outside the object itself which tend to obscure the image. The diaphragm opening must therefore vary with the size of the object under examination and with the power of the microscope, a smaller opening being used with a higher power. This adjustment is accomplished by a revolving disc with openings of different size in the cruder microscopes, and by an Iris diaphragm in more elaborate instruments.

The arm is borne by the upper part of the pillar which moves in the lower part, and this motion, called the fine adjustment, is regulated by a milled head at the top. By this means the distance between the lenses and the object may be varied with great delicacy. More extensive changes in the position of the lenses are made possible by the fact that the tube in which they are fixed slides up and down in the clamp which holds it at the end of the arm. This movement, called the coarse adjustment, may be regulated by hand or by a rack-and-pinion.

The tube itself is divided into two portions, the tube proper and an inner cylinder, the draw-tube, which may be pulled out telescope fashion from its upper end. Into the lower end of the tube proper the various objectives may be screwed, while the eyepieces or oculars slip into the upper end of the draw-tube. When the draw-tube is pulled out the space between the two systems of lenses is increased, and in order that the real image produced by the objective may be visible through the eyepiece, it must be formed farther up than would ordinarily be the case. A reference to Fig. 16 will show that, according to the principles previously deduced, this can be accomplished by bringing the object nearer the principal focus of the objective, and that the image of the object so brought nearer the principal focus will be correspondingly enlarged. Thus pulling out the draw-tube increases the magnification.

At least two sets of lenses accompany a compound microscope, commonly two eyepieces and two or more objectives of different magnifying powers. The lenses of some makers are designated by arbitrary symbols; as a rule, however, they are marked with numerals which indicate what is known as their equivalent focus, or the focal length of a simple converging lens which would produce an image of the same size as that formed by the lens in question. The larger the number the lower will be the power. Thus a 1½-in. eyepiece magnifies less than a 1-in. eyepiece, and a ½-in. objective less than a ⅛-in. objective. The commonest high-power objective is the $\frac{1}{12}$, and the $\frac{1}{16}$ is the highest objective which can be practically constructed. By making use of ultra-violet rays with a wave-length only half that of ordinary light the Zeiss Optical Company has recently been able to construct instruments of much higher power. The lenses in this case are of fused quartz, and the image produced, being of course invisible, is recorded on the photographic plate.

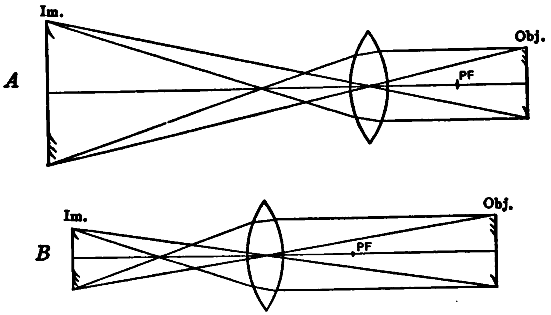

FIG. 16.—RELATION OF DISTANCE FROM PRINCIPAL FOCUS TO SIZE AND POSITION OF IMAGE.

In some microscopes the two lenses of the eyepiece work together to produce the effect shown in Fig. 11. More commonly, however, the real inverted image is formed between these two lenses, and only the upper one forms the virtual image. In such an eyepiece, known as a negative or Huyghenian ocular, the lower or field lens really forms part of the objective system.

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GAGE, S. H. The Microscope. Ithaca, N. Y., 1904.
HAGER, H., and MEZ, C. Das Mikroskop und seine Anwendung. Berlin, 1899.

These references, and those appended to other chapters, include only those few books which have been found most indispensable in the work of the course in Industrial Microscopy as given at the Institute of Technology. A very full bibliography of works on microscopy will be found in Professor Gage's book cited above.

CHAPTER II.

THE MANIPULATION OF THE MICROSCOPE.

1. Setting Up the Microscope.—The microscope is an instrument of precision whose delicacy may be easily impaired by carelessness or neglect. When not in use it should be protected from dust by placing it in its case or under a bell-glass, and any particles which settle upon it should be removed with a camel's-hair brush and chamois-skin. If necessary, the mechanical parts may be first cleaned with chamois-leather moistened in a solution of equal parts of benzine and olive-oil and then wiped with dry chamois.

The microscope should be handled always by the base, and not by the upper part of the pillar, in order to avoid straining the fine adjustment. When in use it should be placed on the work-table rather near the edge, with the pillar side nearest the observer, who should sit close to the table in a chair of such height that his eyes will be a little above the level of the upper end of the draw-tube (see Fig. 17). Those unfamiliar with the microscope are apt to sit too far off or too high above the instrument and bend painfully toward it. For general work it is best not to tilt the tube of the microscope even when the pillar is provided with a joint for that purpose; and if the instrument is kept upright, the spring clips may be dispensed with, the object being moved about on the stage as desired.

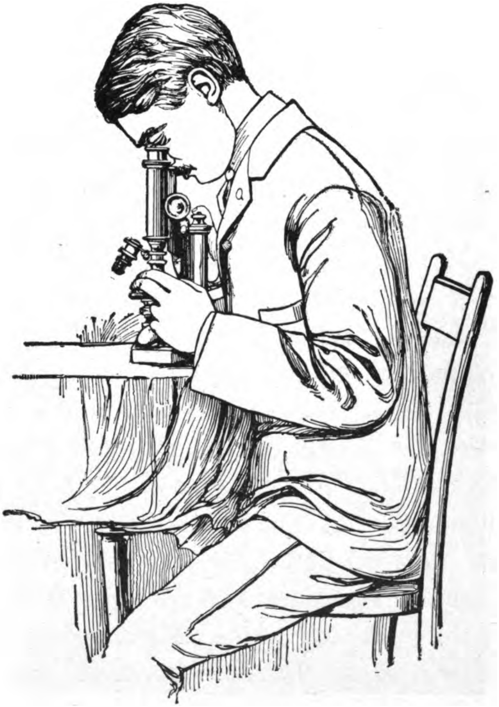

FIG. 17.—PROPER POSITION FOR OBSERVER. (After Bausch.)

The lenses of the microscope should always be examined before they are inserted in order to see if they require cleaning. Since the glass is easily scratched, only soft clean cloth or, better still, Japanese lens-paper is suitable for this purpose; the fingers, which are always oily, must never be allowed to touch the lens. Well-defined spots seen on looking through the microscope at a clear field are due to specks of dust upon the eyepiece, as may be proved by rotating that system of lenses. Not infrequently such specks are formed on the upper surface of the lower or field lens of the ocular, which must be unscrewed in order to remove them. Diffuse cloudiness is generally caused by dirt or moisture on the objective; and this may be removed by breathing on the glass and wiping it with lens-paper. Sometimes for stubborn smears of dirt or grease, 95% alcohol or xylol may be used, but in the latter case the solvent must be sparingly applied and promptly removed in order that it may not affect the setting of the lenses.

2. **Lighting.**—After the objective has been screwed in at the bottom of the tube, as shown in Fig. 18, and the eyepiece has been inserted at the upper end of the draw-tube, the mirror should be so adjusted as to give the best available illumination. Direct sunlight must of course be avoided, but a clear area of sky or a white cloud furnishes an ideal source of light; and sunlight reflected from a white wall or transmitted directly through a curtain may be used to advantage. For artificial illumination electric bulbs with ground-glass shades are suitable, or the narrow edge of the flame from a flat-wicked oil-lamp may be substituted. A gas-flame, unless some incandescent mantle be attached to the burner, is not satisfactory on account of its unsteadiness.

The flat surface of the mirror yields sufficient light under ordinary circumstances, but the concave surface may be used when the strongest illumination is desirable. The observer should so adjust its angle in relation to the window or lamp that a clear and well-defined circle of light may be seen.

If the light be uneven, or if an image of the window-bars or the flame be visible, either the angle of the mirror or its distance from the stage may be so changed as to remove the difficulty. If the circle is too bright so that its glare hurts the eyes, the angle of the mirror must be changed to moderate it and the diaphragm opening should be decreased.

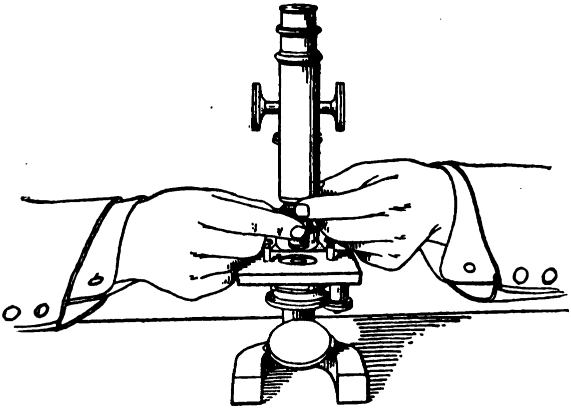

FIG. 18.—METHOD OF INSERTING OBJECTIVES. (After Bausch.)

For most purposes it is desirable that the light should pass directly upward through the axis of the microscope—axial illumination. This end may be attained by focusing just below an air-bubble, and so arranging the mirror that the bright point inside shall be exactly at the center of the bubble. In examining diatoms and some other objects, oblique illumination brings out certain structures best. Light so oblique that it cannot enter the objective at all is sometimes obtained by placing under the stage a stop cutting off the central cone of light. In such a preparation the object will be made visible by the rays which it reflects or refracts upward, and will appear self-luminous on a black background—dark-ground illumination.

With objectives higher than ⅛ in. even the concave mirror will not give sufficient light for successful microscopic work, and the Abbé condenser must be used, with the plane mirror. The condenser, as has been stated in Chapter I, is an objective system of lenses placed just under the stage in such a position as to concentrate a considerable amount of light upon the object to be examined (Fig. 19). With low powers the condenser should be swung out from its place to one side; while with the 1/12-in. immersion objective the best results may be obtained by placing a drop of oil between the condenser and the bottom of the slide, making the entire system optically homogeneous. When one is working with these high magnifications it is also important that the condenser should be accurately centered and focussed; that is, it must be at such a distance below the stage that the greatest possible amount of light may be concentrated upon the object. This position may be determined by focusing upon some specimen with a ⅔-in. objective and then so adjusting the condenser that the image of a window-sash or of a flame coincides with the object itself.

It has been pointed out that the function of the diaphragm is to cut off adventitious light so that all the rays finally reaching the microscope shall come from the immediate vicinity of the object. A little experimentation will show that reducing the diaphragm opening often gives a sharper picture as well as one much less trying to the eyes; in general an opening about the size of the front lens of the objective will yield good results.

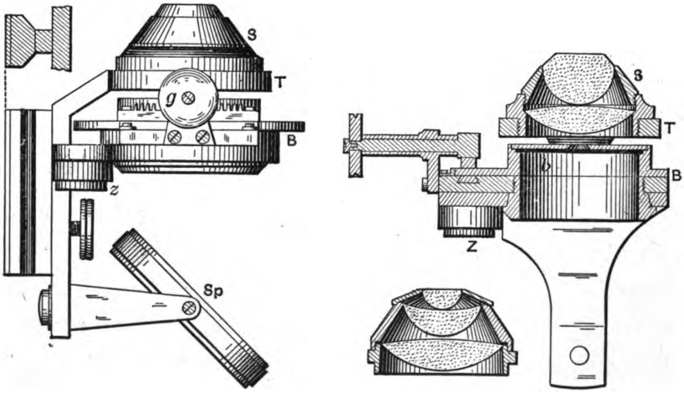

FIG. 19.—ABBÈ CONDENSER. (After Hager-Mez.)

Certain opaque objects are best examined by reflected light, and in many cases this method may well be used to supplement the ordinary one, the mirror being turned aside so that no light passes through the stage and the object being illuminated either with the light which naturally falls on it or with rays concentrated from above by a lens or mirror.

3. **Focusing.**—After the field is well lighted, the next step is to focus on the object, or to so adjust the relation of the lens systems that a clear image may be formed.

The low-power objectives (those of one-half inch and one-third inch equivalent focus) should always be used for preliminary exploration before a new specimen is examined with the higher powers, since their large field of view makes the general relations of the object clear. Focusing is also much easier with the lower powers, since with them a small object may easily be found, while their working distance, or the space between the objective and the object when in focus, is great enough to allow free play. After placing the object over the center of the opening of the stage, the tube should be run down by means of the coarse adjustment until the front lens is within an eighth of an inch of the object. Then, looking through the microscope, the tube is slowly raised by the coarse adjustment until the object comes more or less clearly into view. It is often helpful to move the slide about with the left hand at the same time, as the shadowy outline of a moving object is more readily recognized than that of one at rest.

As soon as the object is dimly seen it may be brought clearly into focus by the use of the fine adjustment. The proper manipulation of this mechanism is of great importance, since if the attempt be made to study objects which are not well in focus, the eyes will be strained and structures incorrectly seen. Furthermore, at any given position of the fine adjustment only a certain plane of the object examined will be in focus, while its whole figure is needed to make up a correct picture. Therefore, one hand should be kept continually on the fine adjustment while the microscope is in use, varying it slightly as needs demand. It should be noted that the excursion of the fine adjustment is necessarily small; if care is not taken to keep it near the middle of its range, its motion will be stopped short at one end or it will be unscrewed from its place at the other.

Focusing with the higher power is more difficult. On account of the short working distance it is necessary to run the objective down as close to the object as possible, watching its approach from the side, and then focusing up very carefully with the fine adjustment.

Useful practice in focusing may be obtained by the study of air-bubbles and oil-drops. A few drops of clove-oil should be mixed with mucilage and the mixture beaten up on a slide with a knife-blade so as to insure the inclusion of air-bubbles; the preparation is then covered with a cover-slip and examined. At first glance the air-bubbles and oil-bubbles appear alike as black rings with lighter centers. Focusing carefully downward, however, it will be found that the air-bubbles appear first as a dark ring with a diffuse lighter center,

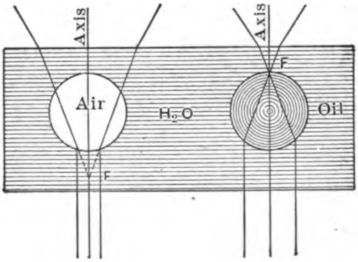

FIG. 20.—REFRACTION BY AIR-BUBBLES AND OIL-DROPS. (After Gage.)

the ring widening and the center becoming sharper as the objective passes downward. With the oil-bubble this is reversed, the brightest center and the widest, ring being above. Why this is so will be seen from Fig. 18. The oil-bubble, a dense sphere in a less dense medium, acts like an ordinary lens concentrating light to a bright focus above, the surrounding ring from which the light is diverted being dark. This dark ring will widen upward. The air-bubble, on the other hand, being less dense than the mucilage, diverges the rays of light, and its dark ring is widest below.

4. **The Use of the Draw-tube.**—The spherical and chromatic aberration of an objective vary with its relation to the object examined and to the eyepiece. Therefore when we say that in an achromatic objective these defects have been corrected, this is only true for a certain standard set of conditions. If we change the position of the draw-tube and therefore the distance between the objective and eyepiece systems, a certain amount of aberration is again introduced. For each objective there is a certain proper tube-length, record of which accompanies the microscope and may be placed upon the objective itself. According to the best American practice the tube-length, measured between the upper end of the tube where the eyepiece is inserted and the lower end of the tube where the objective is inserted, is either 160 or 216 mm.

A cover-glass is commonly placed between the object and the objective in order to hold the former in place and to protect the latter; and this introduces a perturbation which must be allowed for. Reference to Fig. 21 will show in a general way how a cover-glass shifts the rays coming from a point, *F*, and makes them arise apparently from *F'* and *F''*. In the making of achromatic objectives this effect of the cover-glass is taken into account; but with each lens, standard results can only be obtained with cover-glasses of a certain thickness. A variation of .05 mm. in thickness may quite obliterate certain fine structures. Adjustable objectives are so made that by turning a ring or collar the distance between their systems of lenses may be varied, being increased for the thinner cover-glasses. With an ordinary unadjustable objective one may select cover-glasses of the thickness for which it is corrected, the dimension being easily measured by some such apparatus, as is shown in Fig. 22. Or the lens system may be adjusted for cover-glasses other than the standard by altering the position of the draw-tube, changes in the tube-length producing changes similar to those which are provided for in the adjustable objective. The tube-length should be increased for thin covers and decreased for those thicker than the

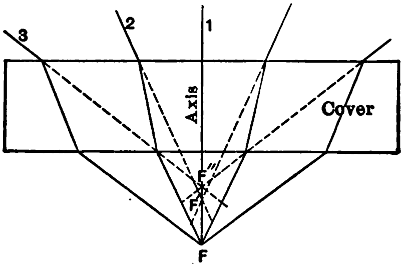

FIG. 21.—DISTURBING EFFECT OF THE COVER-GLASS. (After Gage.)

standard. Such changes in adjustment are necessary only in the case of delicate work.

5. **Care of the Eyes.**—Much of the difficulty sometimes experienced by beginners with the microscope may be avoided by attention to three points mentioned above—the cleanliness of the lenses, a clear but not excessive illumination of the field, and a proper manipulation of the fine adjustment. In no case should the observer strain his eyes in the attempt to study what he cannot see clearly. If the object is not distinct, there must be something wrong which should be remedied.

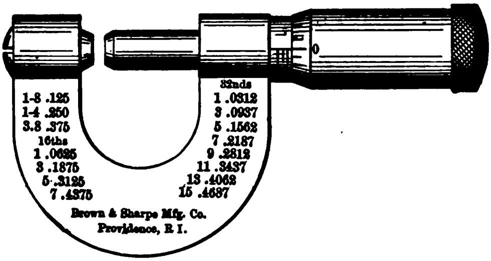
1-8 .125
1-4 .250
3.8 .375
16ths
1 .0625
3 .1875
5 .3125
7 .4375
32nds
1 .0312
3 .0937
5 .1562
7 .2187
9 .2812
11 .3437
13 .4062
15 .4687
Brown & Sharpe Mfg. Co.
Providence, R. I.
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FIG. 22.—MICROMETER CALIPERS.

The distance of the eye above the eyepiece should vary with the magnification. For each combination of lenses there is a certain eye-point at which a maximum number of rays is most closely concentrated, and above or below, the size of the field will be reduced, and colored shadows will appear. The higher the power the nearer the eye-point approaches to the eyepiece.

It is best to accustom oneself to the use of both eyes alternately, and to acquire the habit of keeping open the eye which is not over the instrument. At first the attention may be distracted by external objects, but this difficulty will pass with practice. The beginner may also be misled by seeing through the microscope certain cloudy specks floating across the field of view. These are the muscæ volitantes, shreds of matter lying in the vitreous humor of the eye; after a time they are so discounted by the observer that he becomes unconscious of their presence.

6. Qualifications of a Good Microscope.—The principal parts of the compound microscope have been described in Chapter I; it remains only to point out some of the most important qualities by which we may measure the value of any individual instrument. Differences in the mechanical parts are largely a matter of personal preference, although the stand should be as compact as possible with a base sufficiently heavy to give proper steadiness. Both the coarse and fine adjustments must work easily without being so loose as to allow the tube to slip down of its own weight. The fine adjustment should have an ample excursion. The mirror-bar ought to move freely, and yet retain any position in which it is placed. The stage should be wide enough to accommodate large objects when necessary.

The optical parts are, of course, of prime importance; they should be examined with respect to the four qualities of magnifying power, resolving power, penetration, and illuminating power. The magnification of a lens, or the ratio between the size of an object and the size of the image formed, depends simply on the curvature of its surfaces and may be measured by the method described in Chapter IV. The resolving power by which fine structures are made visible varies directly with the numerical aperture, as has been already explained. This power is ordinarily tested by examining the wing-scales of certain Lepidoptera or the shells of diatoms which possess very fine markings. For example, the shell of Pleurosigma shows three systems of striations when examined under a magnification of 250 diameters with a numerical aperture of over .80, while two of them disappear with lesser aperture. Penetration, or the power to see clearly different planes of the object at the same time, varies inversely with the numerical aperture and directly with the square of the equivalent focus of the lens. Illuminating power varies with the square of the numerical aperture, and with the square of the equivalent focus.

Two common faults should be looked for in a microscope, curvature of the field and imperfect correction of chromatic and spherical aberration. If a stage micrometer, marked with lines at right angles, be examined, the image should resemble *a*, Fig. 23. If it has the appearance of *b* or *c* the lenses are so ground that the magnification is greater or less at the periphery than at the center.

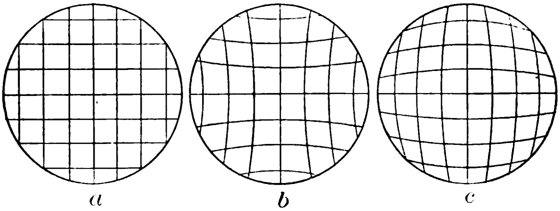

FIG. 23.—CURVATURE OF THE FIELD. (After Hager-Mez.)

Spherical aberration may be detected by examining one of the test objects mentioned above. If the lens be under-corrected, so that peripheral rays come to a focus nearer the lens, the outer part of the object will be in focus in a plane below the central portion. Chromatic aberration may be detected in the examination of any object by the appearance of colored rings surrounding it.

7. Interpretation of Appearances.—The greatest care must be taken in drawing conclusions as to the real structure of bodies from their appearance under the microscope. Right and left are reversed by the inverting action of the objective lenses. Appearances in any one plane may be very deceptive, as in the case of an air-bubble at its upper edge. The medium in which an object is mounted may completely alter its appearance, as we shall see in Chapter III. It must always be remembered that contrasts of density are what give us microscopic pictures; only when these coincide with salient differences in structure will such pictures be representative. The presence of dust or other foreign materials may mislead the observer, and even air-bubbles have sometimes caused confusion. If the preparation has been treated with fixing agents, dyes, etc., as described in Chapter III, great care must be exercised not to mistake artificial conditions due to reagents for those normally existing.

Motion of bodies under the microscope is particularly deceptive. Since the distance travelled is magnified, an idea of rapid movement is conveyed when the actual translation may be really very slow. Diffusion currents are often set up in the mounting medium which may be misinterpreted. A still more serious source of error lies in the phenomenon known as the Brownian movement, or pedesis, a dancing, oscillating motion which affects suspensions of finely divided solid particles under certain not very clearly understood conditions. Gamboge or carmine suspended in water shows the Brownian movement well, and should be studied carefully in order that such motion may not be confused with that characteristic of the bacteria or other living organisms.

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CHAPTER III.

MOUNTING AND PREPARATION OF OBJECTS FOR THE MICROSCOPE.

1. **The Effect of Mounting Media.**—The clearness with which we see an object depends in great part upon the contrast between its density or color and the background furnished by its surroundings. Under the microscope little remains but difference in density to emphasize outlines, and the distinctness of a given object will thus vary widely according to the medium in which it lies. The student may obtain an idea of the importance of this factor and of the necessity for taking it into account in interpreting microscopic appearances, by the examination of potato-starch grains in air, water, glycerin, and clove-oil. In air the grains will show heavy black edges and little internal structure. In water the edges are less pronounced and the hilum and oyster-shell markings appear. In glycerin the same characteristics are still more pronounced, and in clove-oil the edges are almost invisible, so that the grains have a spectral appearance and the hila are very strongly marked. Obviously, it is necessary in making deductions as to the real structure of an object to consider the influence

of the mounting medium; and it is also desirable by choosing a medium of proper density to minimize as far as possible the errors due to excessive refraction. Thus, when starch is mounted in air, the difference in refractive index is so great that the heavy black edges produced are most deceptive, while in clove-oil the difference is too slight for clear definition.

2. **Temporary Mounting Media.**—When an object is of such nature that it can be well examined in air, it may be placed upon an ordinary glass slide (usually 3 in. by 1 in., best with ground-glass edges) and examined directly under the microscope. Even in this case, however, if the high objectives are used, the specimen should be covered with a cover-slip in order to protect the front lens. Of the cover-glasses commonly sold the square ones are most convenient for ordinary handling, and the round ones best suited for making permanent mounts, as will be seen later. The effect of the thickness of the cover-slip upon microscopic vision has already been considered in Chapter II. In fine work it is often desirable to measure the thickness of the covers used in order to select those of a standard size or to adjust the objective or draw-tube to those which deviate from it. This may be effected conveniently by means of micrometer calipers or with certain special forms of apparatus placed upon the market for this purpose (Fig. 22).

Most objects are more clearly seen in some denser medium than air, and, when mounted in any liquid, a cover-slip is always necessary even for low powers. The choice of a medium must be determined by two considerations. First, as suggested above, its refractive index should be far enough, and yet not too far, removed from that of the object to be examined. Second, it should be of such a nature that it will not set up destructive, osmotic, chemical, or other changes in the substance of the specimen. For aquatic plants and animals and a great many other objects water fulfils these requirements; and for specimens of animal tissue the normal fluids of the body furnish ideal mounting media. With denser objects, where it is necessary to minimize contrast effects, glycerin or some oil is preferable.

3. Principles of Permanent Mounting.—In making permanent mounts of microscopic objects it is necessary to guard against changes due to (*a*) physical displacement of the object, mounting medium, or cover; (*b*) chemical changes in the substance or the mounting medium or loss of the latter by evaporation; and (*c*) bacterial decomposition of the specimen or its substratum. First, then, the cover-glass must be firmly attached to the slide either by an adhesive mounting medium or by the application of a special cement. Second, the medium must be protected from evaporation and made of such a nature that it will not undergo chemical change. Third, the mounting medium must be antiseptic.

These requirements are fulfilled in three quite different ways: by mounting "dry" or in air, in which case the absence of moisture serves as an antiseptic, by mounting in aqueous media mixed with glycerin, and by mounting in balsam, a resin which sets and forms a solid mass impervious to all external agencies.

4. **Mounting Dry or in Air.**—The method of dry mounting is well suited to such objects as crystals, which show well in air, and which may be dried without suffering alteration. It is fairly simple, although in this respect, as well as in permanency, it is excelled by the balsam mount.

For all methods of mounting, thoroughly cleaned slides and covers form a first essential. Slides which have been already used and are soiled with balsam or other resinous substances must first be treated with xylol or turpentine. Otherwise all slides and covers should be put into a cleaning mixture made up by dissolving 20 grams of potassium bichromate in 100 cc. of water and adding 100 cc. of sulphuric acid. From this they may be transferred to 50% alcohol, and in that solution kept until needed for use. Both slides and covers should always be handled by their edges, since the oils of the skin will infallibly soil their surfaces.

Given the slide and the cover, there must be constructed some sort of wall or cell which shall separate the two and prevent the specimen from being crushed. The cell will vary in depth with the thickness of the object, and, when a shallow cell will serve, it is only necessary to make a ring of cement and place a round cover directly upon it. A neat mount may be made by using a turntable such as is shown in Fig. 24, the slide being clamped on the movable portion and whirled about, while a brush dipped in shellac, marine glue, Bell's cement, or some similar material is held just over one of the guide rings on the turntable with tip lightly touching the glass. A narrow, even ring may thus be produced, and, by a gradual process of upbuilding, one of considerable depth. If an object of greater thickness is to be examined, a ring of brass or hard rubber may be attached to the slide by a ring of cement similarly applied.

When the cell has become so dry that there is no danger of the cement running, the object to be mounted may be placed within it or attached to the under surface of a cover-slip. The cover is then pressed gently down on the ring of cement so that contact is complete all the way round, and, after placing the slide on the turntable once more, a fresh ring of cement is applied partly on the edge of the cover and partly on the slide.

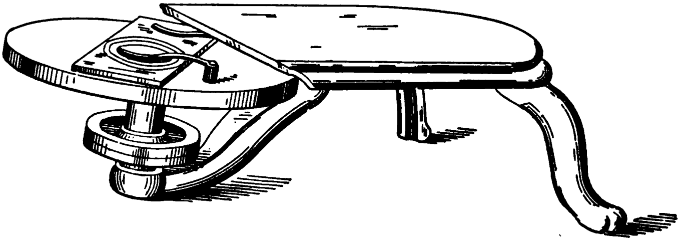

FIG. 24.—TURNTABLE. (After Gage.)

When thus prepared the dry mount is completed and should be labelled on the left-hand end of the slide, the nature of the specimen, the treatment to which it has been subjected, the mounting medium, and the date being indicated.

5. **Mounting in Glycerin Media.** — Glycerin mounts, although not easy to make and less permanent than bal-

sam mounts, are suitable for microscopic plants and other objects which it is desirable to examine without removing all the water which they contain. Some preliminary treatment is necessary even in this case, since cells containing a large amount of water, if placed directly in a strong solution of glycerin, would be torn and distorted by the violent diffusion currents set up. The most convenient way to prepare such objects is to immerse them in 10% glycerin and set them by for a few days under cover, as a protection from dust, so that the water may evaporate and produce a gradual concentration of the glycerin.

Specimens thus prepared may be mounted in a deep cell filled with pure glycerin, but it is a difficult matter to cover the cell so that air-bubbles shall not be included; and a still more difficult matter to cement the cover firmly to the wetted surface of the ring. For most purposes glycerin jelly * is much more convenient, since this substance becomes solid on cooling and obviates the necessity for a cell or for cement to attach the cover-slip. All that is necessary in using this medium is to melt it by gentle warmth, cover the object with a good-sized drop of it, put on the cover-slip, and set aside in a cool place.

6. Mounting in Balsam Media.—The most permanent and satisfactory of all mounting media is Canada balsam, since this substance sets like a rock and has a high index of refraction, which makes opaque objects very distinct. It is commonly used dissolved in xylol, the natural fir balsam being mixed with an equal volume of the oil; the solution after filtering is concentrated to a syrupy consistency by evaporation. It is used like glycerin jelly, being placed on the object, covered and set aside, when the xylol evaporates and the balsam becomes firmly set.

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* One part of gelatin by weight is soaked in 6 parts of water 7 parts of pure glycerin are added and finally 1% of phenol. The mixture is warmed for ten to fifteen minutes, with constant stirring, till it is clear and is then filtered.

With an object of firm texture, not containing water, the preparation of a balsam mount is very simple. The wing or leg of an insect, for example, may easily be mounted in this way. With softer specimens, especially those made up largely of water, it is necessary to resort to some preliminary treatment which shall harden and dehydrate the tissues.

7. **Fixing and Dehydration.**—The first step in the preparation of a soft plant or animal tissue for balsam mounting is to treat it with some agent which shall fix the cell structures in exactly the condition in which they occurred in life, preventing the disintegrating changes which normally follow the death of protoplasm, and which shall at the same time so act chemically upon the cell constituents as to harden and protect them from the action of the chemical agents to be applied later. Flemming's mixture is one of the best of these fixing agents; it contains .25% chromic acid, .1% osmic acid, .1% glacial acetic acid dissolved in water, and should be allowed to act for half an hour or more. A saturated solution of corrosive sublimate is often useful. Absolute alcohol

may be applied to the fixation of many tissues, its action being so rapid as to forestall the bad effect of the violent dehydration which it sets up. At the other extreme, dilute alcohol (1 part 90% alcohol in 2 of water) produces a moderate fixation without harmful osmotic changes.

After fixing with weak alcohol, or after washing out Flemming's fluid or corrosive sublimate with water, the next step is to dehydrate, which can best be done by treatment with alcohol of increasing strength. After the specimen has been thoroughly permeated with 30% alcohol, it should be transferred to a 50% solution, then to 70%, 90%, and 95%, successively. Thus the water is removed so gradually that the diffusion currents set up are not sufficiently violent to distort the tissue. The period of immersion in each grade of alcohol will vary with the thickness of the specimen. For sections or minute objects three to five minutes will suffice.

The last step before mounting an object in balsam is to treat it with a clearing agent, that is, with some liquid of high refractive index which will penetrate its tissues and make them clear, just as glycerin makes starch-grains more transparent than when mounted in air. Since the object is already in alcohol and is to be mounted in balsam, it is obvious that the clearing agent should be miscible with both substances. Cedar-oil, clove-oil, and xylol are perhaps the commonest clearing agents. After being treated with any one of them until it is thoroughly transparent, the object may be placed in balsam and mounted.

The clearing agents just described depend for their action solely upon the fact that their high index of refraction prevents loss of the light passing through the object. In the preparation of certain very opaque botanical specimens, clearing solutions are used which act chemically upon the tissues and actually dissolve certain constituents which interfere with transparency. Of these, strong aqueous solutions of potassium hydrate or chloral hydrate are commonly used, and they must be allowed to act upon the specimen for several hours or even days.

8. Section-cutting.—Since only very small objects can be examined entire, it is necessary in many cases to prepare thin sections of objects for examination with the microscope. Even with fibres and similar objects which can be easily studied in one dimension, cross-sections are often desirable in order to gain an idea of their whole structure.

Sections of rigid objects may be cut directly with a sharp razor; but few specimens are sufficiently hard to be treated in this way. In general, plant and animal tissues are so soft that they would give way even before a sharp knife, and must be supported by imbedding them in some material of firmer texture. If the sections to be cut are not very thin, it is only necessary to surround the specimen as a whole by a substance like pith; if more delicate work is to be done, the tissue must be permeated by some material like paraffin or celloidin which will support each individual cell wall and make it rigid.

Plant stems and leaves, of which sections a tenth of a millimeter or more in thickness will serve, may be easily imbedded and cut in pith. The fresh tissue, without preliminary treatment, is placed in a slit cut at the end of a section of moistened elder pith. A thread is tied around the pith, a little below the end, to hold all together. Then the pith is taken between the thumb and forefinger of the left hand, with the forefinger almost level with the end and the thumb lower down. The knife or razor, held in the right hand, and steadied on the forefinger of the left hand, is drawn toward the body so as to cut off a thin slice of the pith with its imbedded specimen. The section thus cut may then be examined at once in water or prepared in dilute glycerin for a glycerin mount, or passed through the grades of alcohol for the balsam mount.

When thin sections, down to thousandths of a millimeter in thickness, are desired, the object must be imbedded in paraffin or celloidin and cut with a microtome; this is almost always necessary with animal tissues, which are softer than plant tissues, and at the same time more opaque. For imbedding in paraffin, the object must first be fixed, dehydrated, and cleared, as described above. It is then placed in paraffin, kept at a temperature just above its melting-point in a suitably regulated bath, and allowed to remain until thoroughly saturated. The liquid paraffin and the object are then transferred to some sort of temporary cell made of paper, or of metal blocks, in which it can be cooled rapidly by a current of running water. Slow cooling produces crystals of paraffin fatal to clean sections.

Once well imbedded, a cube of solid paraffin containing the object is cut out and fixed in a microtome, such as is illustrated in Fig. 25. Several good instruments are on the market, of which the Thoma and the Minot-Blake types may be mentioned. In any case the knife and object are firmly held in supports moving at right angles to each other; and one or other of the supports is so arranged that by some mechanical device it can be shifted by any desired amount between each excursion, according to the thickness of the sections desired. If the surrounding temperature be right, the successive sections of paraffin will adhere by their edges, forming a long ribbon. In such a ribbon each serial section represents one plane in the original specimen; and the whole object may thus be unrolled as it were upon the slide, each structure being easily traced from section to section.

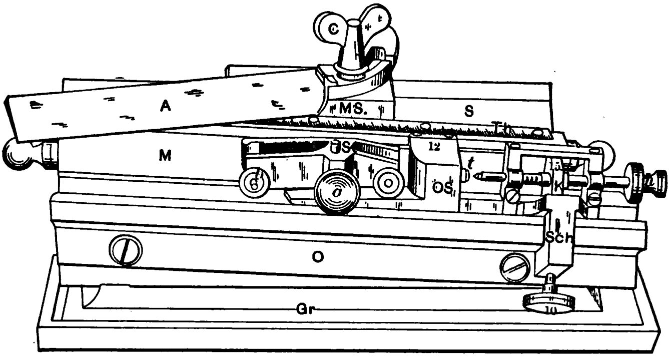

FIG. 25.—THOMA MICROTOME. (After Carpenter-Dallinger.)

Minute sections obviously cannot be handled by themselves. It is desirable, therefore, to affix them to the slide upon which they are to be mounted; and this is best accomplished by the use of Mayer's albumin fixative.* A minute drop of this solution is placed on a clean slide and rubbed with the finger till only the thinnest film remains. The section is then placed upon the slide and gently heated till the paraffin just melts. The section will adhere firmly to the slide and the paraffin may be dissolved in xylol or any other clearing agent.

For objects over 10 mm. in diameter paraffin is unsuitable, since large blocks split under the knife; for such specimens celloidin may be used as an imbedding medium. This substance is used dissolved in ether, and after the evaporation of the latter is hardened by treatment with alcohol or chloroform. The process is less simple than the paraffin procedure, and it is not possible to cut such thin sections of the objects imbedded.

The freezing microtome, in which the water in the specimen acts at a low temperature, as its imbedding substance, is rapid and yields very thin sections. Cell structures are, however, somewhat distorted in this process, and its use is confined mainly to the preparation of pathological material.

9. **Staining.**—One more process in the preparation of objects for the microscope still demands reference—the process of differential staining. Since the elements of a tissue or of a cell differ in chemical composition, it is possible to apply certain dyes which shall enter into combination with some of them and not with others,

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* 50 cc. of the albumin of hen's eggs is mixed with 50 cc. of glycerin and 1 gram of sodium salicylate, shaken well and filtered.

and thus emphasize their outline and bring out the structure more clearly. Sometimes the stain acts upon a certain tissue as a whole, picking it out from other tissues surrounding it. More generally, however, the substances used act on a certain part of the individual cell, generally the nucleus, making that structure stand out clearly from the cytoplasm.

Staining processes are often complicated, and include, not only direct methods in which the stain is allowed to act just long enough to affect the desired elements and then washed out, but also indirect methods in which the tissue is overstained and the dye then removed by alcohol or acid from the parts which give it up most readily. Small objects may be stained in bulk and sections treated on the slide.

Of the staining solutions used, hæmatein and the anilin dyes (fuchsin, methylene blue, eosin, safranin, Bismarck brown, etc.) are the most generally useful. By a proper combination of two of these it is possible to stain the cytoplasm of the cell one color and the nuclei another. This process of double staining depends of course on the greater avidity of the nuclear substance for most dyes. A good example for practice in double staining is the hæmatein-eosin combination. The section, attached to the slide with albumin fixative and cleared, is passed down through the grades of alcohol to 30%, and is then placed for one or two minutes in a solution of Mayer's hæmalum.* The section is then washed in water till the stain is removed from the cytoplasm and passed through 30%, 50%, and 70% alcohol. At this stage it is immersed for a few seconds in a solution of eosin in 70% alcohol to stain the cytoplasm, and is then passed rapidly up through 90% and 95% alcohol and xylol to be mounted in balsam. The nuclei should be dark blue and the cytoplasm reddish yellow.

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* Mix 1 gram hæmatein dissolved in 50 cc. of 90% alcohol with 50 grams alum dissolved in 1000 cc. water. Cool, settle, and filter

Another good stain for the beginner to use, though not to make up, is the Wright modification of Leishman's blood-stain, which with a single solution yields a most beautiful differentiation of half a dozen cell elements. Its use is described more fully in Chapter IX.

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CHAPTER IV.

MICROMETRY AND THE CAMERA LUCIDA.

1. **The Stage Micrometer.**—Objects may be directly measured under the simple microscope by the use of dividers, whose spread is determined, under the lens, by means of a steel scale divided into fifths of a millimeter. With higher powers, some special standard is required; and glass slides bearing fine lines ruled at intervals of .1 and .01 mm. are prepared for this purpose. These are known as stage micrometers. The unit in which the measurement of microscopic objects is commonly expressed is .001 mm., known as the micromillimeter or micron, and denoted by the Greek letter μ.

Obviously such a scale is the only absolute essential for micrometry or measurement with the microscope, and it would be possible to find the dimensions of an object by simply placing it on the ruled slide and reading off the number of spaces covered. This process is, however, open to two objections. Accurately graduated stage micrometers, which are expensive, would be too subject to breakage to make their employment in this manner economical. Furthermore, on such a scale it is difficult to measure small objects, since, as mounted on the slide, they tend to lie obliquely across the scale, and their edges would only rarely coincide with its lines. As a rule, therefore, some indirect method of measurement is adopted.

2. **The Ocular Micrometer.**—The most satisfactory method of measurement with the microscope involves the use of another scale, called the ocular micrometer. This is a circular piece of glass cut to fit in the eyepiece of the microscope and bearing a scale made up of lines at an equal, but not necessarily a known, distance apart. The micrometer is to be placed inside the eyepiece, resting on the sliding diaphragm inside it, which should be so adjusted as to lie in the plane of the real image formed by the objective. Thus the image will lie on the scale, and may be measured by it. The value observed is therefore the size of the virtual image of an object formed by the objective in terms of an arbitrary scale. In order to obtain actual dimensions the ocular micrometer must be standardized in order to determine the value of its divisions as compared to the image of an object of known size. For this purpose it is simply necessary to place a stage micrometer under the microscope and focus upon it with the ocular micrometer in position. The ratio of ocular divisions to stage divisions (or rather to the images of stage divisions) can then be read off and, knowing the actual value of the stage divisions, it is easy to calculate the number of microns on the stage which correspond to one ocular division. When once the ocular micrometer is standardized for a given system

of lenses and length of draw-tube, the stage micrometer may be discarded.

In using the ocular micrometer it is necessary, as we have seen, that the diaphragm in the eyepiece should be at a proper level or the scale will not be distinct. Too strong a light also blurs the image of the micrometer divisions. The object to be measured and the scale should be arranged in relation to each other, by rotating the eyepiece and adjusting the slide, so that one edge of the object shall coincide with the edge of one of the lines of the scale. Since these lines have a considerable thickness, care must be taken to refer the object to corresponding edges of the lines at its extremities. The difficulty of estimating the boundaries of objects with accuracy is always great; and the limits of precision of measurement in the best microscopic work is about .2 μ.

3. **Measurement with the Camera Lucida.** — Any method by which the image of an object may be compared with the image of a scale of known dimensions will serve for measurement with the microscope; and, although the ocular micrometer is the simplest and most satisfactory process, there are several others, of which one involving the use of the camera lucida may be briefly mentioned. This apparatus, whose external appearance is shown in Fig. 26, fits over the eyepiece of the microscope. It contains a system of mirrors which reflect the rays of light coming from a paper laid beside the microscope, so that they enter the eye along with the rays which pass up through the microscope. Thus the image of the object upon the stage and that of the paper are super-

posed one upon the other. Fig. 27 indicates diagrammatically the general arrangement of the mechanism. At *P*, just over the eyepiece, is a cube made up of two triangular prisms of glass with a silvered surface between them pierced by a central opening. Through this opening the rays from the microscope pass, while those coming from the paper, *AB*, are reflected first from the swinging mirror at the side and then upward from the silvered surface of the prisms. In order to avoid distortion it is necessary that the mirror should be at an angle of 45° to the surface on which the paper rests. In order to bring this about it is necessary to support the paper on an inclined surface when the base of the microscope extends outward so as to interfere with a view of the table.

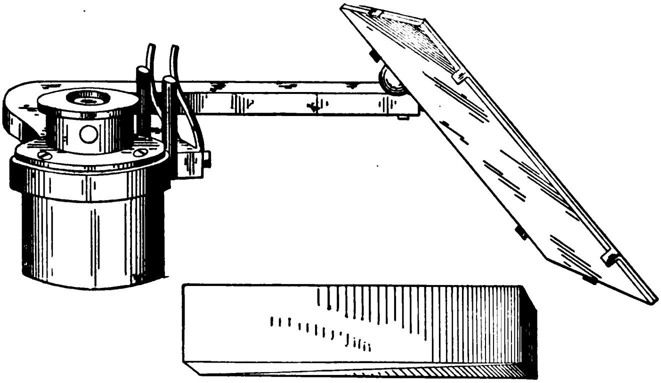

FIG. 26.—CAMERA LUCIDA. (After Gage.)

The adjustment of light with the camera lucida is a matter of considerable difficulty. If the light passing through the microscope be too bright, only the object will be seen, and if, as more commonly occurs, the light from the paper is the strongest, the object becomes almost invisible. When the illumination is properly balanced, both object and paper should be clearly seen, and the point of a pencil on the paper may be made to trace the outline of the object with accuracy. Obviously this device, primarily intended as an aid to the making of drawings of microscopic objects, may be used in micrometry. With a given arrangement of apparatus, the size of the drawing on the paper will depend upon the size of the object. By removing the latter and substituting a stage micrometer whose image will be superposed upon that of the drawing, the size of the original object may be read off directly.

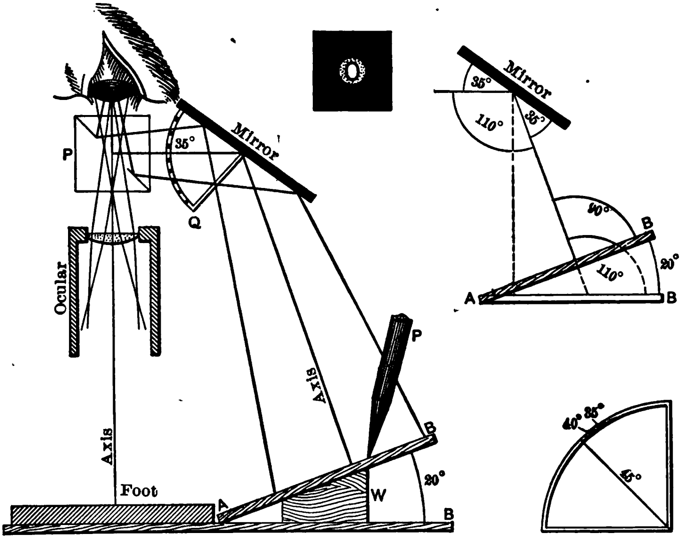

FIG. 27.—COURSE OF RAYS IN THE CAMERA LUCIDA. (After Gage.)

4. Determination of the Magnification of the Microscope.—If the image of a stage micrometer be projected upon paper and drawn in pencil, it will obviously be possible, by comparing the actual size of the drawing with the known dimensions of the micrometer scale, to measure the magnification effected by the microscope. The size of the picture will, however, vary with the distances between the paper and the camera lucida, and it is necessary to adopt for this distance a standard value, generally 250 mm.

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CHAPTER V.

THE MICROSCOPY OF THE COMMON STARCHES.

1. **Origin and Nature of Starch.**—All living things depend for existence upon the potential energy of organic compounds which they take in as foods and break down to simpler substances; and to make good this constant drain upon the limited stock of organic matter in the world there is only one source of supply, the chlorophyll bodies of green plants. Here rays of sunlight are absorbed, and by their energy carbon dioxid and water are united to form starch, oxygen being set free; the kinetic energy of the sun is thus transformed and stored up in potential form. Ultimately the whole structure of organic life depends on this process, as the universe, of mythology, rested upon the back of the fabled tortoise.

Starch is an insoluble carbohydrate substance belonging to the cellulose group and having probably the formula $(C_5H_{10}O_5)_x$. It occurs as a white powder made up of small grains a few hundredths, or rarely a tenth, of a millimeter in diameter, of a shape varying with the plant by which it is formed, but more or less characteristic for each species. The grains are in general built up of concentric layers, the outer one resembling cellulose, while the inner layers contain an increasing proportion of water. At the center there is frequently a small open space of much less density, known as the hilum. Starch may be seen *in situ* in the chlorophyll bodies of many leaves in process of active vegetation; but it is rapidly changed to sugar by the enzymes of the plant, and in that form is conveyed to the root, the tuber, the stem, or any other convenient tissue, where it may be reconverted into starch and stored for future use. The seeds are particularly rich in such reserve material, and with underground roots and stems they furnish the principal sources of starch as a commercial product.

2. **Refining of Starch.**—The processes of starch manufacture are in general simple, involving only the mechanical separation of the grains from the tissue in which they are embedded. Thus, in the production of potato-starch, the tubers after washing are ground up in a comminutor to break the cell walls as far as possible. The milky fluid produced is passed through a sieve with openings about $^1/_{60}$ of an inch in diameter which allow the grains to pass while retaining a large proportion of the pulpy tissue. The starch is then separated from the impurities still present, either by settling, in tanks or in long troughs through which it flows very slowly, or by centrifugal machines; finally it is dried and barrelled for market.

In potatoes the starch makes up perhaps a fifth of the total weight and four-fifths of the dry substance; but in the various grains it is found in combination with nitrogenous compounds, glutens, etc., which somewhat complicate the refining process. Corn-starch may be separated with comparative ease, and is the cheapest of all the starches; but in wheat and rice the union between carbohydrates and albuminoid matter is so close as to introduce serious difficulties. In the latter case it is necessary to use strong alkalies; and with wheat the same end has generally been obtained by moistening and thus swelling the grain, bruising it and then allowing it to stand and ferment until the nitrogenous substance has been partly rotted and disintegrated. The unfortunate feature of this method lies in the necessary loss of the glutinous constituents of the grain which possess high nutritive value; in certain processes the fermentation is omitted and gluten and bran are obtained as by-products.

The cost of the various starches depends both on the difficulty of separation and on the value of the raw materials. Its range is indicated by the following list of prices compiled by Dr. H. W. Wiley for the year 1899:

MEAN WHOLESALE PRICE OF VARIOUS STARCHES.

	Cents per pound.
Corn	1.46–1.61
Sago flour	3.73–3.98
Potato	4.21–4.57
Tapioca flour	4.63–4.96
Wheat	5.00–9.00
Rice	7.50–9.00

3. Commercial Uses of Starch. — Wheat-starch was well known to the ancient Greeks and Egyptians, having been first produced, it is said, in Chios, but it was not until the seventeenth century that the potato was used as a source of carbohydrate; and corn-starch is of course a still more recent product. These three are by far the most important of the starches. As might be inferred from the scale of prices given above, corn-starch is used for most purposes, the amount produced in the United States in 1903 being probably in the neighborhood of 75,000 tons. Potato-starch ranks next with a production of 16,000 tons in 1903, and the amount of wheat-starch was perhaps 10,000 tons.

The purposes for which this great supply is intended may be grouped roughly under four heads: starch is used for a food material, for stiffening and sizing, as a powder, or as a raw material for the manufacture of other substances. Mixed with nitrogenous bodies, in the form of flour, its supreme importance is of course as a nutritive substance; and even the purified product, in the case of corn-starch, forms no insignificant contribution to our dietary. The sago and tapioca flours are mainly used for food, and the various arrowroots contain starches recommended as particularly desirable for invalids.

The principal commercial importance of refined starch, however, comes from the fact that when in contact with hot water its grains swell up and burst, forming a thick adhesive paste. This may be best brought about by mixing starch with cold water and slowly pouring the thick milky fluid into boiling water which is meanwhile agitated by constant stirring. The paste thus formed imparts to textile materials a high degree of lustre and that stiffness from which the name of starch (German, Stärke) was originally derived. Its adhesiveness makes it desirable for bookbinder's paste. Its stiffening power gives it supreme importance in the laundry; for society has not yet outgrown what the Puritan divine described as "a certaine kinde of liquide matter which they call starch, wherein the devill hath willed them to wash and drie their ruffles, which when they be dry, will then stand stiffe and inflexible about their necks." The quality of forming a size gives starch an increasing importance in cotton- and paper-mills, print-works and bleacheries.

As a powder, starch is used in many pharmaceutical preparations, in baking-powders and other products where some finely divided neutral substance is desirable, and for powdering the forms in printing-houses.

Finally, starch when heated in the dry condition to 150°–200° F. yields a soluble dextrin, sometimes called British gum, extensively used as a substitute for gum arabic. When treated with weak acids or with certain enzymes starch is converted into dextrins and sugars and may thus serve as the raw material for the manufacture of glucose, maltose, and ultimately of alcohol.

4. **Microscopical Examination of Starch.**—Since the chemical composition of the various starches is identical, the microscope offers the only satisfactory means of studying them. Fortunately the appearance of starch-grains from the various groups of plants is quite characteristic; and one familiar with the commoner forms can easily detect the sophistication of other substances, like mustard, by means of starch, the presence of foreign bodies (minerals or seed-hulls) in starch, or the adul-

teration of one kind of starch with another cheaper variety.

Being finely powdered, starch is in a condition admirably adapted for microscopic examination, and needs only to be mounted in some medium which will set off its characteristics by proper contrast. If examined dry, the edges of the grains appear so black as to obscure the view, and most solid mounting media have on the other hand so high an index of refraction that except with polarized light the starch becomes too faint. Water with ordinary illumination gives a good picture; and if the hilum is to be particularly studied, a medium of higher density like clove-oil may be useful. Sometimes the detection of starch may be aided by staining it with a dilute solution of iodine, which produces the blue-black color of the iodo-starch reaction.

5. **Potato-starch.**—Potato-starch is produced in considerable amount both in New England and in certain Western States, and is used mainly in print-works for the sizing of warp yarn before it is woven. Other starches make a more even and permanent paste, as a rule, but for this particular purpose potato-starch seems best adapted.

As viewed with a hand-lens potato-starch may be at once distinguished from most other varieties by the fact that a mass of it appears to be studded with glittering points, while corn-starch or wheat-starch is of a dull dead white. Under the microscope potato-starch is seen to be made up of large grains, .05–.12 mm. long, of a flattened ovoid shape, with a smooth and regular outline. As in all starches, there is a considerable variation both in shape and size; many grains are ellipsoidal, some three-cornered; and the smaller ones almost spherical. Near one end of the grain the hilum appears as a well-marked dot, surrounded by rather faint concentric ellipses which form an important characteristic of potato-starch and are known as the oyster-shell markings. Sometimes two or more hila appear in a single grain, and often groups of two or three grains seem to grow together, as shown in Fig. 28 (5).

6. **Wheat-starch.**—Wheat-starch makes a very even paste and, in spite of its cost, is used in many processes where especially fine work is necessary. It is mixed with the colors in printing cloth and is utilized in the bleaching, dyeing, and finishing processes. It is also largely used in paper-mills.

Microscopically, the grains of wheat-starch usually appear as somewhat irregular circles, but when tipped up on edge their true shape is seen to be lenticular. In size they vary from very minute points up to .04 mm., and it is noticeable that the grains are mainly of two sizes, quite large and quite small, intermediate grades being rarer (Fig. 28 (1)). The hilum can sometimes be made out in the largest grains; under the same conditions concentric circles may indicate the natural layers of the grain, though as a rule neither hilum nor markings are apparent.

7. **Corn-starch.**—Corn-starch, as we have seen, is in America by far the most important of starches, being used in the kitchen, the laundry, and the mill for diverse purposes, as well as for the manufacture of dextrins and

sugars. It may often be found as an adulterant in the more costly starches, and in spices and other foods.

Its grain is easily recognized, being of medium size, .01–.02 mm., and of a characteristic polyhedral form, its angularity at once distinguishing it from the starches of wheat and potato. Since it is not flattened like the latter,

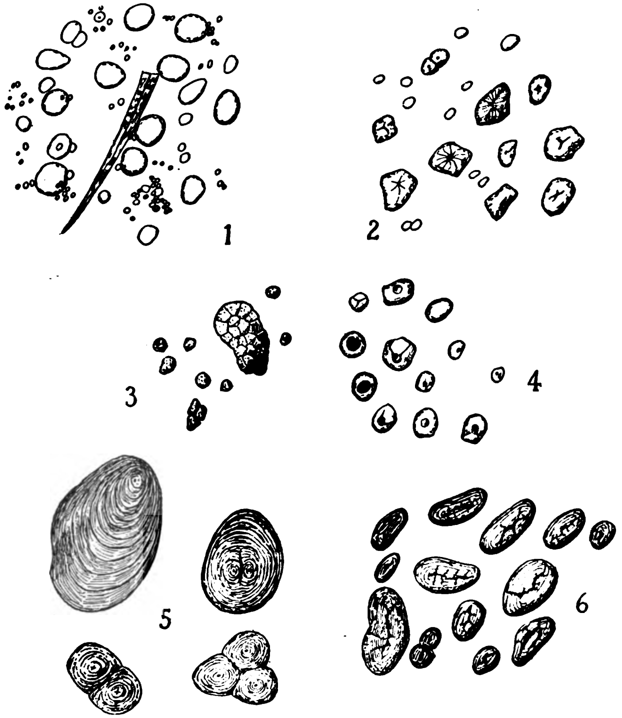

FIG. 28.—THE COMMONER STARCHES. (Redrawn, after Schimper.) 240 diameters.

1. Wheat-starch.
2. Corn-starch.
3. Rice-starch.
4. Tapioca-starch.
5. Potato-starch.
6. Bean-starch.

but approximately isodiametric, it shows very black edges in air- and water-mounts. Concentric layers are absent; but the hilum is sharply marked, showing in water-mount the form of a cross with cracks radiating out toward the periphery of the grain. In denser media this effect disappears and the hilum is seen to be round (Fig. 28 (2)).

8. Rice-starch.—Rice-starch resembles that of corn in a general way, being polyhedral in shape with distinct facets and angles. It is very much smaller, however, having an average diameter of about .004–.008 mm., and the grains are rather regular in size. Another characteristic is found in the fact that the grains commonly occur aggregated in masses. As a rule no hilum can be seen (Fig. 28 (3)). This starch is used to some extent as an adulterant, and as a constituent of various powders.

9. The Starches of the Pea and Bean.—The pea and bean, as well as certain other plants of the order Leguminosæ, have starches of a very characteristic type; and though not of special importance in themselves, an acquaintance with their appearance is an important aid in the detection of adulteration with the ground-seeds of these species. Bean-starch, which may be taken as an example of this group, has grains of an elliptical or kidney shape with a size of .02–.06 mm. The hilum has the form of a slit running the long way of the grain, while distinct concentric layers may be made out in a good light (Fig. 28 (6)).

10. The Arrowroots.—Arrowroot-starches are extensively used as foods for invalids and for certain other special purposes, and are rather liable to sophistication. The characters of the group vary widely, and we can only consider one common example. Bermuda arrowroot has a starch which in its flattened ovoid shape and the possession of oyster-shell markings resembles that of the potato. The grains are however smaller, .02–.03 mm., and the hilum, usually at the larger end of the grain, is typically elongated to the form of a slit. The smaller grains are not rounded as in potato-starch, but have the same shape as the larger ones.

11. **Sago and the Cassavas.**—Tapioca is derived from the root of the bitter or poisonous cassava, the hydrocyanic acid which it contains being driven off in the process of manufacture. The sweet cassava, in which this substance is not present, yields a similar starch which has recently been introduced into the trade for certain sizing processes to which it is supposed to be specially adapted. Tapioca starch-grains are very smoothly circular and often show a truncated end. The hilum is central and slit-like. The size is .01–.02 mm. (Fig. 28 (4)).

The grains of sago-starch have a similar hilum, and occasionally show truncated ends. They are larger than those of tapioca (.02–.05 mm.), and phenomenally irregular, the roughly ellipsoidal form being generally distorted.

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CHAPTER VI.

FOODS AND DRUGS AND THEIR ADULTERANTS.

1. Microscopical Examination of Foods and Drugs.—In the examination of foods and drugs for the detection of adulterants the microscope is of very great value; and in many cases it furnishes the only satisfactory method of analysis. Mr. A. E. Leach says, in his treatise on "Food Inspection and Analysis": "The chemical constants of many of the adulterants of coffee and the spices do not always differ sufficiently from those of the pure foods in which they appear to be distinguished therefrom with accuracy and confidence by a chemical analysis alone. On the other hand, one who is familiar with the appearance under the microscope of the pure foods, and of the starches and various ground substances used as adulterants, can with certainty identify very minute quantities of these materials, when present, with the same ease that one can recognize megascopically the most familiar objects about him."

The starches, already treated in Chapter V, furnish perhaps the best examples of a case in which foreign materials present may be readily detected under the microscope. The identification of such substances as coffee and mustard, containing a number of complex tissues, is more difficult, but a careful comparison with samples of known purity insures reasonable certainty.

The technique of the microscopical examination is very simple. The substance to be observed is ground up fine (so as to pass through a 60-mesh sieve), further comminuted between the fingers, mounted in water, and examined directly. It is sometimes advisable to rub the powder to a still finer condition by manipulating the cover-glass and slide between the thumb and finger. The process should not be carried too far, because in tissues which are broken up into very fine fragments the characteristic structures are often indistinguishable. Better views of the structure of opaque objects may be obtained by clearing or rendering them transparent by the action of strong alkalies, caustic soda, or chloral hydrate. In practice, however, this is not generally necessary.

The student should be careful that whatever comes in contact with the specimen to be examined is quite clean and free from the contamination of previous specimens. Several samples from different portions of the material should be examined in order to gain an idea of its average composition. Since the recognition of fragments of tissue depends largely upon the personal element, certain points catching the eye of each observer, the comparison with standard pure samples should never be dispensed with.

2. General Nature of Food Adulterants.—A very thorough and systematic examination of foods is carried on by the State Board of Health of Massachusetts; and the annual reports of that board furnish valuable data as to the actual condition of commercial substances. Spices, coffee, and cocoa are the most important foods for which the microscopical analysis is found available; while chocolate, tea, tobacco, allspice, cassia, pepper, cayenne, paprika, cloves, ginger, mustard, nutmeg, vanilla, cardamom, and numerous other materials may be examined to advantage. Cocoa frequently contains foreign cereals, starch, or sugar; coffee is adulterated with pea-hulls, peas, chicory, wheat, and charcoal. On the other hand, in some of the foods advertised as substitutes for coffee a considerable admixture of coffee may be found. Tea, cloves, pepper, and mustard are most frequently adulterated with refuse portions of the plant in question—tea-stems, clove-stems, pepper-shells, and mustard-hulls respectively. Occasionally very bad samples of cloves occur with a large proportion of wheat, turmeric, and charcoal; of mustard, nine-tenths wheat and turmeric; of pepper, one-third olive-stones.

It would be unprofitable, even if it were possible, for the student to attempt to cover the whole field of microscopical analysis, since the detailed information involved can best be acquired in practice when it is needed.

We shall therefore take up only three of the most important substances, coffee, mustard, and pepper, with their commonest adulterants, as types of the rest, and as illustrating the sort of characteristics by which vegetable food substances are identified.

3. **The Microscopic Structure of Coffee.**—The coffee-bean is the seed of *Coffea arabica*, a small tropical tree of the family Rubiaceæ, two of the semi-ellipsoidal beans lying base to base in each of its berries. The beans are brought into the market generally roasted, either in their original form or ground, and although adulteration is most easy in the second condition, artificially modelled beans of foreign material are not unknown.

The true coffee-bean is made of thick-walled cells, approximately isodiametric, and packed with a finely granular material containing minute oil-drops. The cells of the inner part are very characteristic, showing knotty thickenings of their walls, as indicated at *B* in Fig. 29. More peripheral cells (*C*) are smaller and lack these swellings, while at the extreme outside of the bean is the so-called silverskin, a thin glistening layer containing peculiar fusiform cells with wide walls pierced by

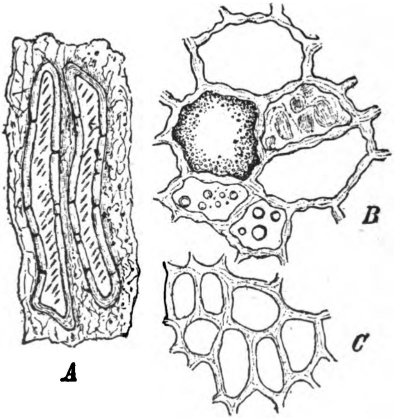

FIG. 29.—MICROSCOPIC STRUCTURE OF COFFEE. (After Schimper.) 240 diameters.

channels (*A*, Fig. 29). The first two tissues appear, as a rule, as large opaque dark-brown masses, with the cell structure showing only at the edge.

The detection under the microscope of other cell structures than those mentioned at once serves to indicate the presence of adulterants. Leach has pointed out that even a naked-eye examination sometimes reveals foreign substances. "The chicory grains are apparent from their dark and somewhat gummy appearance, and can usually be recognized by crushing them between the teeth. Their soft consistency and bitter taste are very distinctive. The dull surface of the outside of the crushed coffee-grains is in marked contrast to the polished appearance of the surface of the broken peas or beans, often to be found as adulterants, while fragments of broken cereal grains are readily distinguished from coffee with a low-power magnifier, though perhaps not easily identified by the eye alone."

4. **Chicory and other Adulterants of Coffee.**—The roasted root of *Cichorium Intybus* is one of the substances most commonly found mixed with coffee. Its presence is at once betrayed by the appearance of masses of tissue made up of elongated cells of the type common to the stems of the higher plants. The outer layers are thin-walled and delicate, while the woody tissue proper contains large fusiform cells marked with fine scar-like cross-hatching, the ladder-cells of the fibro-vascular bundles. Occasionally, too, particles will be found which show the very peculiar branching, tubular structures of a homogeneous dark color, which are known as the milk-tubes.

The general predominance of elongated cells, the presence of ladder-cells and milk-tubes, the absence of opaque masses of dark-brown tissue and of granular cell-contents make the picture very different from that obtained in the case of coffee.

Various other roasted roots, radishes, carrots, etc., fruits such as figs and pears, with grains of diverse sorts are sometimes used as coffee substitutes. In Massachusetts the following have been found: Roasted peas, beans, wheat, rye, oats, chicory, brown bread, pilot-bread, charcoal, red slate, bark, and dried pellets, the latter consisting of ground peas, pea-hulls, and cereals, held together with molasses. In each case the presence of peculiar cells, other than the simple ones characteristic of the coffee-bean, will reveal the sophistication. The ground-up seeds of the Leguminosæ, peas and pea-hulls, for example, are quite commonly present, and may be detected by the starch-grains described on p. 66, and by the outer layers of the hull, which from their parallel structure are known as the palisade cells.

5. **The Microscopic Structure of Mustard.**—Mustard, as it usually comes to the market, is a mixture of the ground seeds of two closely related herbaceous plants, *Brassica* (*Sinapis*) *alba* and *nigra*. The structure, in both cases, is very similar, and if the entire seed be ground up and examined, three distinct cell types will be apparent. On the outside are several layers of large flat transparent thin-walled cells, some rounded and some polygonal, showing in glycerin faint concentric markings about a median area (*c* and *d*, Fig. 30). These epider-

mal layers are difficult to make out clearly. Within them lies a characteristic tissue known as the columnar layer made up of prismatic cells which, as seen under the microscope, are small and polygonal with heavy walls and small central lumens, giving the whole a dotted appearance. In white mustard (*B. alba*) this tissue is yellowish, while in black mustard (*B. nigra*) it is dark brown, determining in each case the general color of the seed (*b*, Fig. 30). Finally, the interior is a tissue of medium-sized, fairly thick-walled cells, packed with a fine, gray-green, granular material containing oil-drops (*a*, Fig. 30).

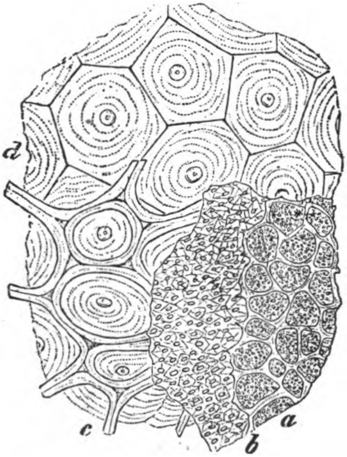

FIG. 30.—MICROSCOPIC STRUCTURE OF MUSTARD. (After Schimper.) 240 diameters.

6. Adulterants of Mustard.—In a good table-mustard the amounts of epidermal and columnar tissue present are very small, since the hulls are largely sifted out; the bulk of the sample should be made up of the inner cell-contents. If an excess of the outer layers be present, it is evident that mustard-hulls have been added; and this is one of the commonest adulterations to which this substance is subjected. Wheat- and rice-starch are also found not uncommonly, and furnish conclusive evidence of sophistication. Yellow aniline dyes are sometimes used to give the proper color to such adulterated samples. Turmeric, which is the ground rhizome of *Curcuma longa*, is also well adapted for the adulteration of mustard. It appears under the microscope in small amorphous, intensely yellow, pasty masses, which stain blue with iodin, being made up largely of curcuma-starch.

7. **The Microscopic Structure of Pepper.**—Pepper is the pulverized seed of *Piper nigrum*, a shrub cultivated mainly in the East Indian islands. The dried seed is about 5 millimeters in diameter and is covered with a brownish hull. If this hull is ground up with the grain, we have the ordinary black pepper. White pepper is made by macerating the fruit in water before drying, and detaching the hulls by friction.

In ground black pepper a number of tissues may be made out, since the many-layered hulls are relatively thick as compared with the more homogeneous central portion. The latter forms the bulk of the preparation, and is quite characteristic in appearance. It consists of irregularly angular whitish masses, seen to be made up of polygonal cells packed full of very minute starch-grains (*s* and *ss*, Fig. 31). With a high power these grains themselves appear polygonal and closely aggregated into masses, while their size is very small, averaging about .003 mm. Besides these starch masses at least two characteristics kinds of cells from the hull may appear in black pepper. The outermost layer furnishes dark-brown fragments made up of the stone-cells so called, polygonal, thick-walled cells with a small dark lumen and striations radiating out from it (*a*, Fig. 31).

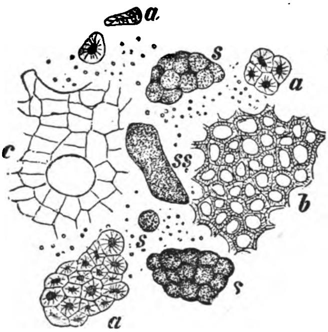

FIG. 31.—MICROSCOPIC STRUCTURE OF PEPPER. (After Schimper.) 240 diameters.

The cells of the inner part of the hull occur in lighter, yellowish-brown masses, and have a somewhat similar structure except that their lumen is much larger and their walls thinner (*b*, Fig. 31.) Smaller fragments of parenchyma tissue may be seen, with irregular, thin-walled cells and occasional large spaces where deposits of oil were present in the fruit (*c*, Fig. 31).

White pepper made up simply of the central part of the seed contains practically nothing but the irregular, silvery masses of pepper-starch with occasional small fragments of the outer tissues.

8. Adulterants of Pepper.—Pepper is one of the most commonly adulterated of spices, and may be found mixed with a wide variety of substances. As in the case of mustard, the ground hulls of the seed itself furnish a frequent sophistication. The most general adulterant in the United States is perhaps buckwheat-starch, which is not easy to detect since it closely resembles that of the pepper in shape and occurrence. The individual grains are, however, about twice the size of the pepper-starch, and the masses, as a rule, are also larger. In Germany dried and ground bread is often used, as well as the bark of trees, bran, sawdust, pulverized nut-shells, and hulls of mustard, rape, peanut, linseed, or almonds. In France and in this country ground olive-stones are a common adulterant. They are made up principally of large fusiform stone-cells which resemble those of the pepper except in their size and the fact that they are practically colorless. The occurrence of any substance other than the normal tissues of the pepper, and in particular of foreign starches and the elongated cells and tracheids characteristic of plant-stems, will suggest the presence of some adulterant.

In all these instances only careful comparison with a series of known pure substances will enable the analyst to determine certainly which of these foreign materials is present.

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CHAPTER VII.

THE EXAMINATION OF TEXTILE FIBRES.

1. **The Kinds of Textile Fibres.**—The word fibre is derived from the Latin *fibra*, and signifies "a thread or filament." The most primitive application of natural fibrous materials to textile purposes is probably in the use of grasses and osiers for weaving. At a very early period, however, primitive man learned to manipulate fibres of a closer texture in the manufacture of coarse cloth. The long, fine hairs of plants and animals among many races must early have attracted attention; thus the application of cotton and wool dates back from beyond recorded history. In Egypt the use of the fibrous bast-layer from the stem of the flax-plant appears to have antedated even that of cotton. According to Chinese tradition, the application of the secretion of the silkworm to textile purposes was made by the first empress of the nation. To-day, four classes of fibres—cotton, the principal vegetable hair; flax, hemp, jute, ramie, and sisal, all typical bast-fibres; wool and a few less well known animal hairs; and silk—still make up the textiles of most importance. The leaves of certain plants furnish filaments essentially similar to the bast-fibres mentioned

above. The leathery covering of the cocoanut supplies a fibre used for matting, etc. Asbestos illustrates the possibility of using a mineral substance for textile purposes. Finally, various artificial fibres are made from metals and from cellulose derivatives.

In the identification of textile fibres the microscope is of prime importance. Animal fibres in general may be distinguished from those of vegetable origin by the fact that both wool and silk are soluble in 5% caustic soda, while the vegetable fibres, made up of cellulose, are not thus affected. Various color reactions are also of value. For the determination of individual fibres, however, the microscope is most satisfactory, since even of the closely related bast-fibres each has its characteristic appearance. Furthermore, the quality of different samples of the same kind of fibre is evidenced by the size, twisting, and external structure of the filaments. The effect of chemical reagents and the tensile strength of individual fibres under various conditions is studied with the aid of the microscope in modern textile laboratories.

2. The Cotton-fibre. — The cotton-fibre is the vegetable hair borne on the seed of plants of the genus *Gossypium*, serving like the down of the thistle for the distribution of the seeds by wind. These plants belong to the Mallow family; some are herbaceous and others grow to be bushes twenty feet in height. As the seed-capsule opens a rich white boll bursts out, made up of the cottonseed with the fibres at its free end.

The cotton-plant grows well between 45° north and

35° south latitude, given proper soil and a uniform supply of moisture. The United States, India, Egypt, China, and Brazil are the most important cotton-producing nations in the order named; though many other countries devote large areas to its cultivation.

The most important process in the preparation of cotton for the market is ginning, or the separation of the fibre from the seed-cotton, which as picked contains two-thirds of its weight in seeds. The modern process, evolved from that which Eli Whitney was largely instrumental in developing in 1794, consists in exposing the cotton to the action of a series of fine-toothed circular saws which tear off the fibre and carry it away through the grid in which they revolve.

The fibre, as thus obtained, is a hollow ribbon (Fig. 32) spirally twisted at frequent intervals with the edges of the ribbon so sharply marked off from the central canal that they appear like swollen cords. The section of the fibre is not, however, dumbbell-shaped, as this might suggest, but elliptical or somewhat crescentic, the lumen of the canal following the outer contour. The canal is narrow in American and Egyptian cotton, and broader in that from India. By following a single fibre carefully along, it will be noticed that it tapers to a somewhat blunt point at one end and at the other extremity is broken off sharply where it was attached to the seed. The length of the cotton fibre varies from 20 to 40 mm.,—its diameter, from 10 to 20 μ.

The cotton-fibre is mainly cellulose, covered with a fine cuticula of different composition. When treated with a solution of hydrate of copper in ammonia, the cellulose dissolves and swells up the cuticula into bubbles between the twists of the fibre, while at the twists the cuticula contracts, giving the characteristic beaded appearance shown in Fig. 31, *e*.

Much of the cotton in textile goods has been subjected to the process of mercerization, or immersion of the cloth in a stretched condition in strong caustic soda solution. Cloth thus treated is stronger, takes dyes better, and has a closer, firmer texture and a fine glossy appearance. Under the microscope the individual fibres appear as crinkly, irregular cylinders instead of flat ribbons and the central canal is much shrunken. Sometimes the fibres are so

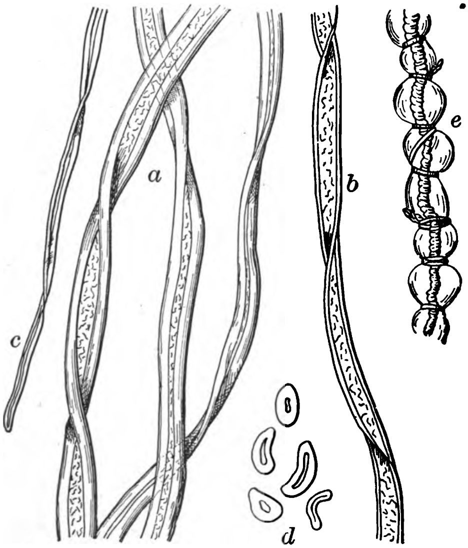

FIG. 32.—THE COTTON-FIBRE. (After Hassack.) 200 diameters.

swollen that even the twists cannot easily be seen in a water-mount; they show more clearly, however, in air.

3. Bast-fibres in General.—Most of the vegetable fibres in commercial use are derived from the bast-layer of the dicotyledonous flowering plants.

The bast, or, as it is called by botanists, the phloem, is a fibrous layer lying just under the bark, and since it is more or less developed in all the higher plants, it would be theoretically possible to obtain textile fibres from any one of them. In preparing the fibre from those herbaceous plants actually in commercial use, the stems are first retted or allowed to ferment under water so that the gummy substances which hold the tissues together may be dissolved. Next they are scutched or exposed to the action of beaters which break up the outer and inner friable tissues, leaving the elongated bast-cells adhering together in threads or bundles. The bast-fibre, as we find it in commerce, is thus made up of a group of cells, not of a single cell like the cotton-fibre.

When further broken up under the microscope, the bast-cell appears as a more or less elongated spindle with a central canal where the living protoplasm once lay, the cell wall of cellulose only being left. Both ends are symmetrically pointed. In size, proportions, cross-section, canal, and markings the bast-fibres of various plants may be distinguished. We shall consider briefly the six fibres which have most commercial importance—flax, hemp, Manila hemp, jute, ramie, and sisal.

4. Flax.—Flax is obtained from the stem of *Linum usitatissimum*, a tall herb widely cultivated in the central regions of Europe and America. The seed of the plant furnishes linseed-oil, and the bast yields flax to be spun into linen. The flax-fibre like others of its class, is a bundle of cells, each a hollow cylinder tapering at both ends (Fig. 33). Its cells are distinguished by their large size (25 to 30 mm. long by .02 mm. in diameter), and by the fact that they are swollen or knotted at frequent intervals. The central canal is narrow and nearly circular in section, while the cell itself is somewhat flattened. Thus when a number of cells are examined under the microscope some will appear much broader than others, while the canal in all of them is narrow. At the end the flax cell tapers gradually to a very sharp point, and the canal disappears at some distance from the tip.

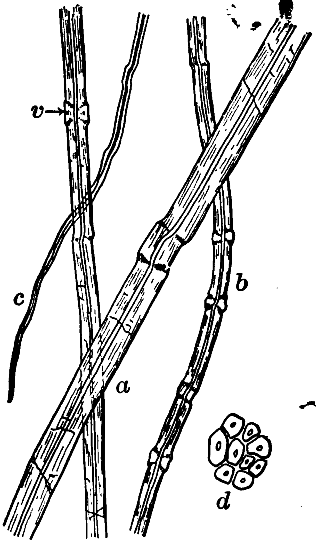

FIG. 33.—THE FLAX-FIBRE. (After Hassack.) 200 diameters.

5. **Hemp.**—The true hemp-fibre, to be distinguished from Manila hemp and Sisal hemp, which will be considered later, is derived from *Cannabis sativa*, an herb with much the same range as the flax-plant, grown most extensively, perhaps, in Russia and Italy. It is a strong fibre, though less pliable than flax, and is used for cordage, sail-making, and the manufacture of certain other textiles. The *Cannabis* plant also yields the drug hashish.

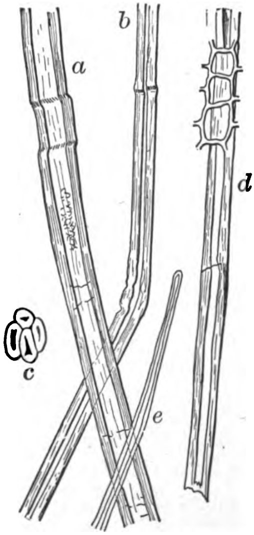

FIG. 34.—THE HEMP-FIBRE. (After Hassack.) 200 diameters.

The hemp-fibre cell very closely resembles that of flax in its microscopic appearance, being of the same size and general shape and showing the same knots and swellings. It may be distinguished by two characteristics. First, the canal, like the cell, is elliptical in cross-section, so that while the cell on its narrow surface cannot be distinguished from flax, its broad diameter shows the broad canal quite distinctly. Second, the ends of the cell are comparatively blunt, and the canal runs up to the very tip (Fig. 34).

6. Jute.—The jute-plant, *Corchorus capsularis,* is an annual, native to the East Indies, and furnishes a long smooth fibre which, however, quickly softens and breaks when wetted. It is used for making rope, coarse twine, and gunny-bags, and for the backing of various other textile materials.

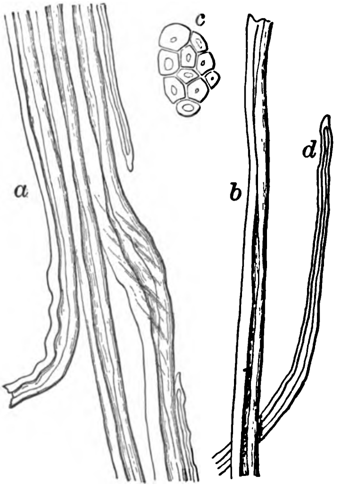

Fig. 35.—The Jute-fibre. (After Hassack.) 200 diameters.

The cells of which the long jute-fibre is made up are much shorter than those of flax and hemp, being only about 2 mm. in length, while their width is nearly the same as that of the other fibres. In any given field of the microscope numerous ends of cells will be apparent, while with the long cells of *Linum* and *Cannabis* the tips are hard to find.

The most marked peculiarity of the jute cell is, however, its canal, which varies markedly in diameter, at one point occupying a large part of the cell and then shrinking to an almost invisible line (Fig. 35).

7. **Ramie.**—Ramie or China grass is the product of a low shrub, ***Bœhmeria nivea***, indigenous to China, Japan, and the Philippine Islands. Its fibre is long and lustrous, stronger probably than any other fibre; it is used for making sail-cloth and for other special purposes. The cells of ramie (Fig. 36) are easily distinguished by their great length, sometimes reaching 50 cm., and by their flat ribbon-like structure. In cross-section they are

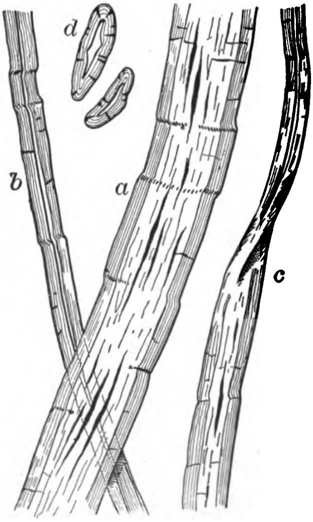

FIG. 36.—RAMIE. (After Hassack.) 200 diameters.

pointed ellipses, .03–.06 × .01–.02 mm., with a wide canal of the same shape as the cell. Under the microscope, therefore, as one sees the two views, ramie appears like a mixture of a broad fibre with a broad canal and a narrow fibre with a narrow canal.

8. **Manila Hemp.**—Bast-fibres are not confined to the stems of plants, but are continued upward in the fibro-vascular bundles which form the veins of the leaves. In many plants these leaf-fibres are of such a character as to be valuable for textile purposes, notably in the case of Manila hemp and Sisal hemp.

Manila hemp is obtained from the leaves of *Musa textilis*, a palm native to the Philippine Islands and North Borneo. The fibre is extracted by hand, and, though more brittle than true hemp, is extensively used for marine cordage. The cells are distinguished from those of *Cannabis* by their lesser length (about 6 mm.) and by their smoothness, the knots, though present, being much less marked than in the hemp-fibres. The canal is wide and easily discerned.

9. **Sisal Hemp.**—Sisal hemp is obtained from the leaves of a cactus, *Agave rigida*, extensively cultivated in Mexico, Central America, and the West Indies. It is a long coarse white fibre used for cordage and the manufacture of rough sackcloth, hammocks, etc.

The cells are distinguished from all other bast-fibres by their smoothness and regularity. They are 1.5–4 mm. long and 20–32 μ wide, approximately cylindrical, with a wide and well-marked canal, and tapering to a fine point at the end. As in other cases, comparison with material of known composition furnishes the only sure criterion for identification.

10. Wool and Other Animal Hairs.—Animal hairs are much more complex structures than those of plants, being not only multicellular, but composed of several distinct layers of cells. In the growth of a hair the epidermis or outer tissue of the skin is first folded in to form a minute pit called the hair-follicle. From the base of this follicle the hair grows out, its cells dividing off from the epidermal tissue below. The hair itself is made up of three distinct zones. In the center is an axis of irregularly rounded cells known as the medullary layer. Outside this, and forming the main portion of the hair, is the cortex, which consists of elongated spindle-shaped cells; and at the periphery is a cuticular layer of flat overlapping scales covering the hair like the shingles on a roof. In the cortical cells is situated the pigment which gives to the hair its color, and to the disintegration of these cells and the formation of air-spaces in their place is due the whitening of the hair in old age.

Externally the principal characteristic of the animal hair is the presence of the scales of the cuticle which give the fibre the effect of being marked with fine transverse, anastomosing lines and which at its edge (Fig. 37) appear as fine serrations. The medullary cells may just be made out beneath this cuticle as fine longitudinal striations.

The general term wool is applied to hairs which are spirally twisted so that when woven they will hold together to form a strong fabric. In such hairs, too, the scales are large and project prominently from the fibre, so that they interlock with each other and materially increase the compactness of the tissue. Some thirty-two varieties of sheep (belonging to the genus *Ovis*) furnish the wool of commerce besides the llama, or alpaca goat, the Thibet or Cashmere goat, and the Angora goat, from which mohair is derived. The length of wool varies from 2 to 20 cm., and its diameter from 10 to 100 μ; the larger fibres are combed and spun into worsted yarns, the shorter are carded and spun into woolen yarns.

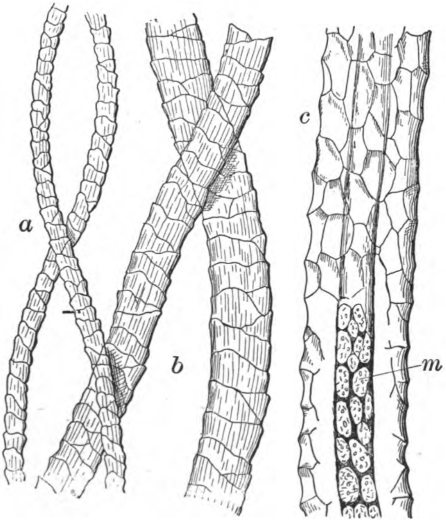

FIG. 37.—WOOL-FIBRES. (After Hassack.) 200 diameters.

The separation of wool from vegetable fibres under the lens is sufficiently simple; but the application of the microscope only begins here. The hair from various animals should be carefully studied; comparison of sheep's wool with camel's hair will prove instructive, showing the lesser length of the latter, its freedom from twisting, the scales lying almost flat against the surface, and the granular spots in the medulla. Different grades of wool may then be studied, contrasting the high-grade merino wools with a diameter of 15 μ and 10 or more twists in every centimeter of length with a wool of poor quality having perhaps four or five times that diameter and not more than one twist in a centimeter. These two qualities, fineness and convolution, principally determine the quality of wool, although its regularity and the projection of its scaly covering are also of importance. A sample of wool shoddy may profitably be examined; various foreign fibres, dyed fibres, and torn and broken fibres will be apparent.

11. **Silk.**—One other animal fibre of quite a different character remains to be considered, the secretion of the silkworm. The larvæ of many moths spin their cocoons out of threads long and strong enough to be used for textile purposes, but that of *Bombyx mori* is cultivated most extensively for commercial purposes in China, Japan, India, France, and Italy.

The substance of which silk is composed is poured out in a liquid condition from two glands at the anterior end of the worm, and when discharged in the air at once sets in the form of solid structureless rods. The pair of fibres formed by the two large spinnerets are cemented together by an incomplete cuticula of somewhat different composition produced by another set of glands and known as sericin. Under the microscope such a double thread at first sight appears like a broad fibre with a central canal (Fig. 38, *a*). In preparing silk for the market, however, the cocoons, before unwinding, are placed in hot water to kill the chrysalis and to melt the gummy material which cements all the threads together. In this process the single fibres become separated and each appears only as a semi-transparent cylinder (Fig. 38, *b*) with no internal structure, and no external markings except very fine longitudinal cracks formed as the fresh secretion sets. Flecks of the ruptured cuticula and particles of foreign material which come in contact with it when semi-liquid sometimes adhere to the fibres. The diameter varies considerably from point to point, according to the rate at which the worm was producing its secretion. Perhaps 10–20 μ would be a fair average. Ends, except

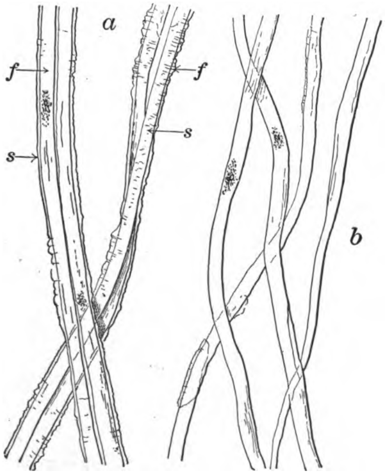

FIG. 38.—SILK. (After Hassack.) 200 diameters.

when produced by accidental breakage, are, of course, absent, since the whole cocoon containing perhaps 500 meters of silk is often made up of a single piece.

12. Analysis of Fabrics.—In the examination of a fabric a sample should be taken 2–3 sq. cm. in size, or large enough to include all the different yarns employed in the pattern. The warp and filling threads are then separated into their constituents, and one of each kind taken for analysis; sometimes there may be a dozen different yarns to examine. Under a low power of the microscope (fifty diameters) the nature of the fibres is determined in the different yarns, taken in the proportion in which they occur in the fabric; a rough quantitative analysis may thus be made.

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CHAPTER VIII.

THE MICROSCOPY OF PAPER.

1. Paper and Paper-making.—The earliest written records were probably made on such natural objects as stones, leaves, bits of bone or wood, or, as in Babylonia and Assyria, upon blocks of clay. A real paper, however, the celebrated papyrus, was manufactured in Egypt many hundred years before the Christian era. The thin, transparent layers of tissue which surround the stem of the papyrus plant were separated with some sharp instrument, superposed under water and then pressed and dried. Large quantities of this product were exported from Alexandria to all parts of Europe and Asia, coming into competition with parchment, prepared by scraping and drying the skin of the sheep and goat.

The modern process of making paper, as a thin layer of cellulose derived from fibrous vegetable material reduced to a pulp in water, was first discovered by the Chinese and introduced into Europe by the Arabs in the eleventh century. Cotton was the first material used for this purpose, but an Arabic manuscript on linen paper bearing the date 1100 is still in existence. The manufacture of paper from wood-pulp appears to have been first suggested by the French naturalist, Réaumur, in

1719, who derived his idea from a study of the way in which wasps construct their nests from this material.

The first step in paper-making is the treatment of the raw stock with some chemical in order to break it up, to dissolve the cementing gums, and to separate the cellulose, which is always the principal constituent of paper, in as pure a form as possible. The crude material is generally boiled with strong alkali under pressure, and then washed in a tank from which the waste water is removed by a revolving drum. Wood paper is also made by an acid process, the stock being treated with the bisulphites of lime and magnesia; and mechanical wood-pulp for a low grade of paper is prepared by simple attrition against stone surfaces without chemical action.

After treatment in the boilers and washers, the paper-stock is bleached, and at this stage is known as "half-stuff." It is next passed through the beaters, in which a wheel bearing knives breaks it up into a fine fibrous condition. Sizing, loading, coloring, and other auxiliary processes are accomplished in the beaters. Finally, the fully prepared pulp passes to the paper-machines, where it is spread in a thin sheet of running water over a moving endless belt of wire cloth. The fibrous material deposited in a fine and even layer passes on through felted rollers, which press out the last of the water and compact its texture.

2. **The Raw Materials of Paper.**—Obviously paper might be made from any material which can be ground up to a fine fibrous pulp; and such bizarre substances as seaweeds, shavings, sawdust, corn-husks, cabbage-

stumps, and leather-cuttings have been used experimentally for this purpose. Cotton and linen rags still furnish the best grade of paper. The manufacture of Manila paper is an important industry. Straw and wood-pulp, however, furnish by far the largest yield of coarse paper and cardboard. The relative importance of the different materials is indicated by the following table from the United States Census:

PAPER-STOCK MANUFACTURED IN THE U. S. IN 1900.

Wood, cords	1,986,310
Wood-fibre, tons	644,006
Straw, tons	367,305
Old paper, tons	356,193
Rags, tons	234,514
Manila, tons	99,301

3. **The Microscopic Examination of Paper.**—Before examining a sample of paper under the microscope, it should be torn into small bits and boiled in a one-percent solution of caustic soda. The wet pulp produced is washed on a fine sieve and broken up by shaking in water.

The identification of paper fibres is by no means an easy task, and requires careful study and comparison with known substances. The student will be much aided by an admirable monograph prepared by Professor W. R. Whitney and Mr. A. G. Woodman, and published in the Technology Quarterly for September, 1902. The authors suggest the following points to be noted in the systematic study of each specimen: size of cells, shape, length, width as compared to length, shape of the ends, presence of knots or joints, whether the majority of the cells are straight or bent or curled, markings, size and character of the central canal, if any. Polarized light is often of great service in the identification of paper fibres.

4. **Analytical Key to Paper Fibres.**—The authors above mentioned suggest the following useful key for the preliminary examination of paper. In this table the word fibre is restricted to those cells which are very long in proportion to their breadth.

ANALYTICAL SCHEME.

A. Fibres are characteristic; other characteristic forms absent.
  I. Fibres are *long;* greater than diameter of field (Mag. = 60).
    (1) Fibre has many joints, knots, or projections, especially by polarized light.
      (*a*) Fibre is fine; quite smooth; joints not very noticeable.
        Paper mulberry—Manila hemp—Agave.
      (*b*) Fibre is coarse; uneven; joints are large and prominent.
        Linen—Jute—Hemp (*Cannabis sativa*).
      (*c*) Fibre shows overlapping scales.
        Wool.
    (2) Fibre has peculiar markings; dotted, circular, square, net-like.
      (*a*) Markings resemble circular or square perforations.
        Spruce (*fir, hemlock, tamarack, balsam*)—*Pine—Redwood—Red cedar—Arbor vitæ—Cypress.*
      (*b*) Markings net-like, "feather-stitch," spiral.
        Redwood — Cypress — Banana (*fruit-stem*) — *Ramie.*
    (3) Fibre is smooth and regular.
      (*a*) Many fibres resemble twisted ribbons.
        Cotton—Banana (*stalk and leaf-stem*).
      (*b*) Fibres are round, cylindrical.
        Silk—Sisal hemp—Bark of cotton-stalk.

II. Fibres are *short;* less than diameter of field (Mag. = 60).
  (1) Ends of fibres frayed and torn.
    Cotton rag—Mechanical wood (conifers).
  (2) Ends of fibres not torn.
    Coir—Elm—Willow.

B. Characteristic forms other than fibres are also present.
  I. Fibres are *long;* equal to or greater than diameter of field (Mag. = 60).
    (1) Fibres quite broad in middle; tapering to point like a bayonet.
      (*a*) One or both ends of cells drawn out, sometimes terminating in a tail.
        Poplar—Birch—Porpor gum.
      (*b*) Ends of cells cut off obliquely at blunt angle.
        Cottonwood—Whitewood—Black walnut—Holly—Chestnut.
    (2) Fibres not varying greatly in width, ends needle-like.
      (*a*) Characteristic forms (cells) *large;* covering ½ to ⅓ diameter of field.
        Bamboo—Sorghum bagasse—Raffia—Tulip.
      (*b*) Characteristic forms *small*, cellular, serrated, or pointed.
        Straw—Esparto—Sugar-cane bagasse—Live Oak.
  II. Fibres are *short;* less than diameter of field (Mag. = 60).
    (1) Cells comparatively long and narrow; quite sharply pointed at one or both ends.
      Magnolia—Tulip—Sweet buckeye.
    (2) Cells prismatic, stubby; ends blunt and cut off obliquely.
      (*a*) Quantities of short, fine, transparent material with square ends.
        Holly—Chestnut.
      (*b*) Field fairly clear except for fibres and characteristic elements.
        Pawpaw — Tree of heaven — Maple — Black cherry—Willow.
    (3) Cells fragmentary, broken; fibres short.
      Groundwood—Elm.

5. The Commoner Paper Fibres.—With a few of the most important materials of paper the student should thoroughly familiarize himself. The fibres of cotton, linen, hemp, manila, jute, and silk have already been treated with some fulness in Chapter VII. Of the woods used in the manufacture of paper pulp there are two distinct types. The Coniferæ—spruce, fir, pine, etc.—exhibit cells of markedly different structure from the Angiosperms, of which poplar and birch are the most important examples. Finally, straw and esparto grass are distinguished by a third characteristic type of tissue.

6. The Structure of the Gymnosperms.—Wood-pulp made from the spruce, fir, balsam, larch, or hemlock is mainly made up of the cells from the tracheids or fibro-vascular bundles of the stem. In all the trees of this group the structure is essentially the same. The cells are long, exceeding the diameter of the low-power field, and of considerable breadth (see Fig. 39). The ends are often contracted to a rather acute point. Sharp twists are sometimes present, due to harsh treatment in the preparation of the fibre. The characteristic feature of these cells, however, is the presence of numerous round

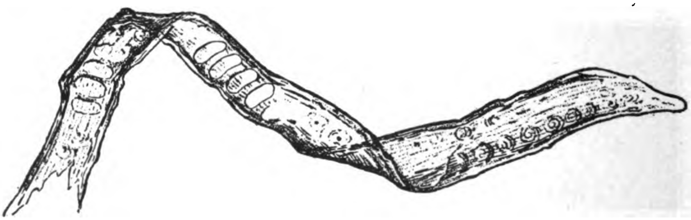

FIG. 39.—TRACHEID OF A CONIFER. (After Herzberg.) 240 diameters.

or oval scar-like pits arranged in regular rows over their surface. In the case of pine, lattice-like areas with oblong openings appear at intervals; but the presence of long cells mainly of a single type and with discoid markings is the characteristic of the Conifers in general.

7. **The Structure of the Angiosperms.**—Pulp made from the commoner Angiospermous trees shows two distinct elements, long narrow fibres and short broad cells with characteristic markings. The two species most in use for cheap grades of paper are poplar and birch; the longer fibres are very similar in both, having a central canal of variable width and ends sometimes rounded and sometimes tapering to a point. The more thin-walled of these fibres often show rounded and oval pores penetrating the wall. Smaller fibres with

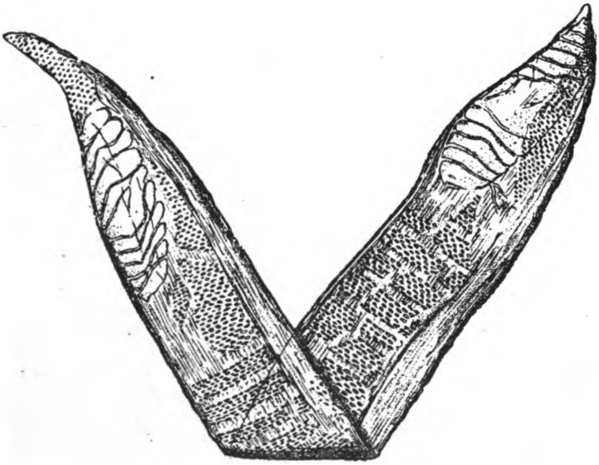

FIG. 40.—TRACHEID OF BIRCH. (After Herzberg.) 240 diameters.

swellings at intervals along their course are also present in birchwood.

The small, broad cells of the fibrovascular bundles are more characteristic. In birch they are very numerous, and are dotted with minute pores arranged in rows at right angles to the long axis of the cell (Fig. 40). The distribution of these pores is somewhat irregular. At the ends of the cell the cross-hatched transverse walls are sometimes seen. In poplar the cells of this type are

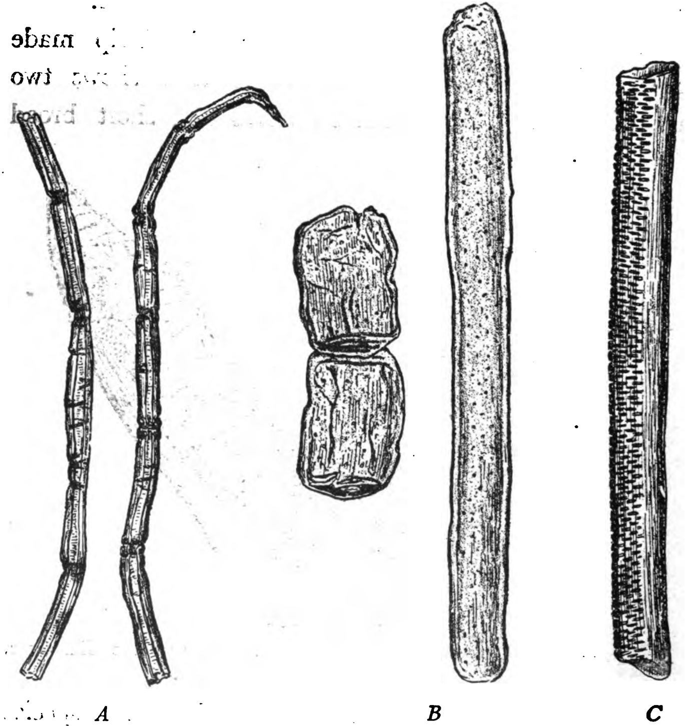

FIG. 41.—CELLS OF STRAW. (After Herzberg.) 240 diameters.

less numerous and show larger pores more closely packed. At the ends of the cells is often a long, tail-like point, and the grating seen in the case of birch is absent.

8. The Fibres of Straw and Esparto.—Paper made from the stems of the grasses and grains is largely made up of long slender bast-fibres knotted or thickened at regular intervals (Fig. 41, *a*). The central canal is constricted at these points and fine pores are present, passing through the wall. The characteristic structures of such tissues are, however, the cells from the epidermis, flat and of somewhat variable length, with thick walls and serrated edges (Fig. 41, *c*).

In straw pulp a third type of cell is present, derived from the internal pith layer. These are large, oval, thin-walled cells with rounded ends (Fig. 41, *b*). Esparto or alfalfa grass (*Stipa tenacissima*) may be distinguished from straw by the absence of these pith cells, and by the smaller size of the bast and epidermal elements.

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CHAPTER IX.

THE MICROSCOPE IN MEDICINE AND SANITATION.

1. **The Microscope in Biology.**—The microscope bears to biology much the same relation that the balance bears to quantitative chemistry. It is the fundamental instrument without which a true science of living things would be almost impossible.

The invention of the compound microscope and the construction of simple microscopes of high power at the middle of the seventeenth century stimulated a dozen observers to the study of the finer structures of plants and animals. Leeuwenhoek discovered in water and in decomposing organic matter a teeming world of minute forms of life invisible to the naked eye. Hooke, the English botanist, made out the cellular structure of plants in his examination of cork. Malpighi and Swammerdam figured the microscopic details of the insect body with marvellous patience and success.

It was only, however, after the perfection of the achromatic objective about 1835 that these early labors could bear abundant fruit. In 1838–39 Schleiden and Schwann developed that great generalization which bears their name, the doctrine that all plants and animals are built up of minute individual masses called cells. Quickly the essential identity of the substance forming the cells was postulated. The microscope thus grounded anatomy upon the firm basis of the cell theory; and to-day cytology, the study of the minuter structure of the cell itself, forms an independent subject of ever-increasing importance. Next it was discovered that the cell is not only the unit of adult structure, but the form in which every living organism originates from its parent. In the study of the course of development by which the single cell is transformed into the mature organism, carried out by Kölliker, Hertwig, Van Beneden, and their compeers, embryology came into being. Upon the anatomical and embryological unity thus demonstrated under the microscope, the doctrine of evolution, the most important scientific contribution of the nineteenth century, was largely founded.

2. The Microscope in Medicine and Sanitary Science.—Of all the branches of biology, none owes more to the compound microscope than the study of disease. The conception of the body as a complex of protoplasmic cells, whose normal coöperation was the condition for good health, led easily to the conclusion that disease might arise from the deranged functioning of the individual cell. Cellular pathology, associated with the name of Virchow, led along this line to great advances in the knowledge of those diseased conditions which arise from the abnormal activity of the living protoplasm.

Another group of maladies due to the invasion of parasitic plants and animals, the epidemic plagues, fevers, and pestilences, was comprehended only after their exciting causes were discovered by the microscope. The bacteria, observed by Leeuwenhoek in 1680, were first carefully studied by Ehrenberg in the wonderful decade of progress which followed the perfection of the achromatic objective. Twenty years later Pasteur established the casual relation of these minute fungi to disease; and in the next quarter of a century the organisms causing anthrax, tuberculosis, typhoid fever, diphtheria, and cholera were discovered. These bacterial parasites are studied largely by culture methods; but the microscope is also indispensable. The animal organism causing malaria cannot be grown on artificial media, and our whole knowledge of it depends on optical methods. In the first few years of the twentieth century, the brilliant researches which revealed the parasites associated with smallpox and scarlet fever were carried out with the microscope alone.

The researches into pure science which have led, and are leading, to such advances in pathology and parasitology cannot, of course, be treated here in detail. Certain applications of the microscope to the study of the body fluids and of food material have, however, become a routine part of clinical diagnosis and the sanitary inspection service. These technical applications of medical microscopy come legitimately within the scope of our work; and a few typical methods for the study of pathologic conditions and of parasitic invaders will now be briefly considered.

3. The Examination of Urine.—Many organic diseases affect materially the composition of the urinary secretion. Its amount, reaction, and specific gravity are significant, and chemical tests for sugar, albumin, etc., are of much value. The presence of certain important organized elements is determined under the microscope.

The precipitate which forms in any urine on standing must be first concentrated by sedimentation, or better, by the use of the centrifuge, transferred by a pipette to a clean slide, covered, and examined with the high power. The edges of the preparation, where evaporation is taking place, should be avoided, since the crystals which form here are not characteristic.

The principal objects which may be found in an examination of urinary sediments are crystals of certain products of metabolism, red and white blood-cells, epithelial cells, and tube-casts. The inorganic salts precipitated may be diverse in character, including uric acid (clusters of rhombic prisms and whetstone forms), acid urates (amorphous, granular masses soluble on warming), calcium oxalate (small octahedral crystals whose diagonal planes look like the edges on the back of an envelope), and ammonium-magnesium phosphate (long prisms with bevelled edges, known as the coffin-shaped crystals). (See Fig. 42.) In hyperemia, acute nephritis, and some other conditions, red blood-corpuscles appear in the urine as homogeneous, yellowish discs 7.5 μ in diameter, sometimes distorted to a globular form or shrivelled and crenated almost beyond recognition. An arbitrary line is drawn between "normal" cells retaining their red color and abnormal colorless and crenated cells. The other formed elements of the blood, the white corpuscles (polymorphonuclear leucocytes), are larger bodies, more or less spherical and possessing several nuclei; the latter may be brought out clearly and differentiated from the cells of the renal epithelium by allowing dilute acetic acid to run under the cover-slip when the nuclei of the two types of cell may be made out. The characteristics

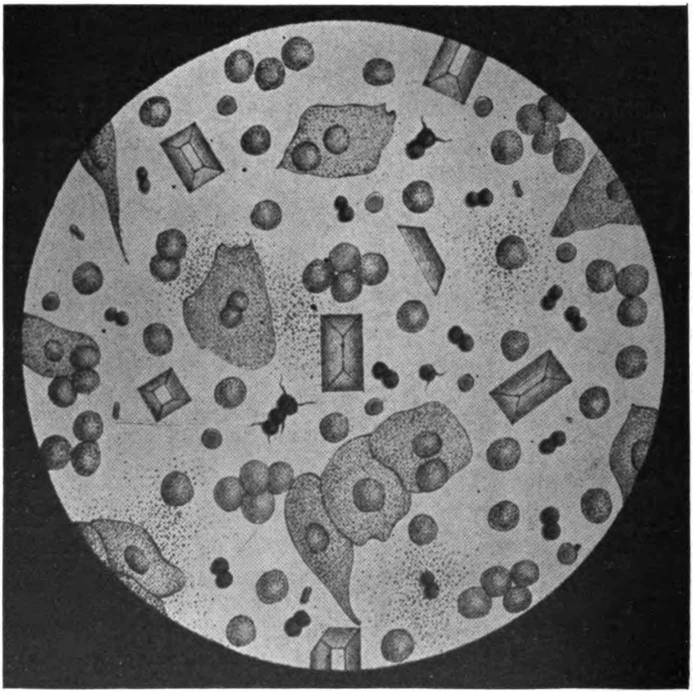

Fig. 42.—Urinary Sediment in Catarrh of the Bladder. (Acid urates, ammonium-magnesium phosphate, leucocytes and epithelial cells.) (After Ultzmann and Hofmann.) 200 diameters.

of the white corpuscles will be treated somewhat more fully in the next section; but their presence in urine is of much significance since it is these cells which mainly make up the whitish pus discharged from inflamed surfaces. When the walls of the genito-urinary tract are seriously affected, cells detached from its lining epithelium will be noted in the urine. The appearance of some of these elements is indicated in Fig. 42. Finally, most renal diseases are characterized by the deposit in the fine tubules of the kidney of an albuminous substance which coagulates to form minute twisted tubes (Fig. 43). These casts found in the urine may be clear and hyaline or may be rendered granular by the presence of disintegrated epithelial cells or may contain undecomposed epithelial cells or pus cells or fat globules or bacteria according to the special pathologic condition from which they arise. So-called waxy casts are denser and more sharply marked than any other type and indicate an advanced stage of renal disease.

4. **Examination of Blood.**—The blood is a colorless fluid or plasma containing cells of several distinct types whose number and relative proportions vary in many physiologic and pathologic states. Few clinical tests are of more value to the physician both in diagnosis and prognosis than the examination of this body fluid under the microscope.

For the study of the various types of cells the blood must be dried and stained; and the preparation of a good blood smear for staining is a matter of some delicacy. The blood may be obtained from the lobe of the ear by pricking with a sterile lance or glover's needle after carefully washing the surface of the skin. A small drop should be caught on the edge of a clean slide, which is then brought in contact with the surface of a second clean slide. The upper glass is drawn along the lower one at an acute angle so that the blood-drop escapes beneath its edges and is spread over the lower slide in a thin

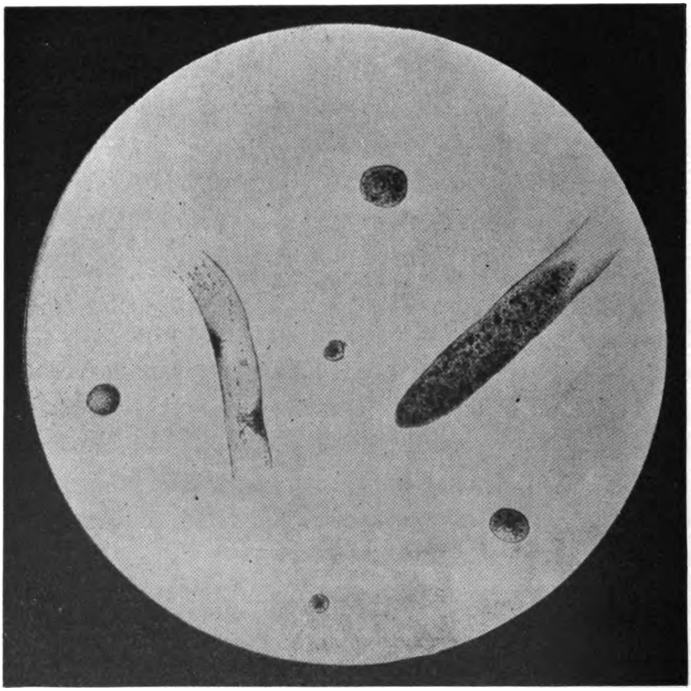

FIG. 43.—URINARY SEDIMENT IN ACUTE BRIGHT'S DISEASE. (Granular casts, leucocytes, and epithelial cells.) (After Ultzmann and Hofmann.) 200 diameters.

even smear. After drying in the air this is ready for staining.

One of the most satisfactory differential blood-stains is Wright's modification of Leishman's method, described in the *Journal of Medical Research* for January, 1902 (volume VII, p. 138). The somewhat complicated procedure for making this stain and some discussion of the philosophy of its action must be sought in the original paper; but the student will find its application easy. The dried film is covered with the solution of the dye in methyl alcohol for one minute and water is added, drop by drop, until the mixture becomes semi-translucent and a yellowish metallic scum forms on the surface. This mixture is allowed to stand on the preparation for two or three minutes and then washed off in distilled water till the film has a yellowish or pinkish tint in its thinner portions.

The slides thus stained, dried, and mounted in balsam should show the various types of blood-cells beautifully differentiated. The red corpuscles, or erythrocytes, are orange or pink in color and about 7.5 μ in diameter. The white cells may be divided into three classes according to the relation which their nuclei and granules bear to the constituents of the stain. The eosinophiles or oxyphiles are cells containing matter which takes such acid stains as eosin. Stained with the Wright stain, these appear faintly blue with a dark lilac-colored nucleus and numerous large reddish granules. They are double the size of the erythrocytes. The basophiles are cells which take basic stains and are of two varieties. Small basophiles or lymphocytes are about the size of the red corpuscles, robin's egg blue in color with a round dark-blue nucleus and a few fine dark-blue granules. Large mononuclear basophiles are three times the size of the red cells and pale blue with a large oval blue nucleus. The third type, the neutrophiles, are intermediate between the red-granuled eosinophiles and the blue-granuled basophiles. They are polymorphonuclear, twice the size of the erythrocytes, and blue, with one or more darker lilac-colored nuclei of a twisted shape and numerous medium-sized granules of a reddish-lilac color. Blood-plates are almost always seen, purplish rounded or oval bodies one-third the size of the erythrocytes, with irregular edges and fine blue mottlings. In pathologic conditions numerous other cell elements may be present. Most of these cell elements are seen in Fig. 44.

Such a stained preparation as that described is used for the study of the structure of the blood-cells and for the determination of the relative number of white cells of the various types. A differential blood count, as it is called, is made by counting several hundred white cells and calculating the percentage of each form. For thus determining the absolute number of the blood corpuscles the apparatus called a hæmocytometer is designed. It consists of a glass slide containing a cell of known diameter and a depth of 0.1 mm., on the bottom of which is ruled a micrometer scale marking off squares 0.025 mm. on a side. The blood is diluted, in a special pipette constructed for the purpose, with 100 parts of Gower's solution (100 cc. water, 15 cc. acetic acid, 6 g. sodium sulphate), when red cells are to be counted. For counting white cells the blood is diluted with 10 parts of ½% acetic acid tinted with gentian violet, which destroys the red cells and faintly stains the white corpuscles.

Wide variations of the blood elements, both in absolute numbers and in proportions, occur in many normal and abnormal conditions. The blood of the adult human being should contain 5,000,000 erythrocytes per cubic millimeter in the male and 4,500,000 in the female and 3000 to 10,000 white blood-cells, of which 70–72% should be polymorphonuclear neutrophiles, 25–30% basophiles, and 1% eosinophiles. In anæmia the red cells may fall to 360,000 per cubic millimeter and in other conditions they may much exceed the normal. Leucocytosis, or the in-

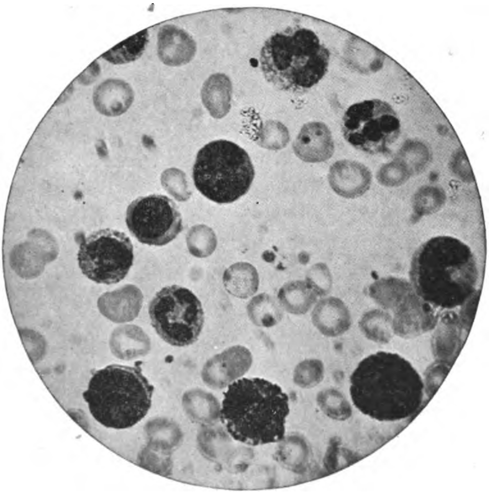

FIG. 44.—BLOOD-CELLS (WRIGHT'S STAINS). (Photomicrograph by W. C. Greene, Harvard Medical School.) (Erythrocytes, large basophiles, polymorphonuclear neutrophiles, and blood plates.) 700 diameters.

crease of white cells, accompanies all acute inflammations as well as certain other conditions and may affect any one type of cells or all of them. In inflammations it is the polymorphonuclear neutrophiles which are increased; in trichinosis, on the other hand, the eosinophiles may reach 50% of the total.

5. The Detection of the Malarial Parasite.—Blood examinations are also undertaken for the diagnosis of malaria. The Protozöon malarial parasites gain entrance to the body from the bite of an infected mosquito in the form of minute spore-like bodies which penetrate the red corpuscles and there assume a crescentic form. Later the organism becomes amœboid, sends out blunt pseudopodia, and develops nuclear granules. As it matures, it occupies the greater part of the red corpuscle and finally begins to divide, appearing in a rosette form, at last separating and discharging into the blood stream granules like those with which the cycle began.

Blood may be examined in its fresh condition for the malarial parasite, but better preparations are obtained by staining. The Wright-Leishman method yields admirable results, the cytoplasm of the parasite being stained blue and the nuclear granules a color varying from lilac through red to almost black. The general appearance of blood-cells containing the parasite of tertian malaria, *Hæmamœba vivax*, is indicated in the two upper fields of Fig. 45; the lower field shows a later flagellated stage in the flood stream. Only considerable experience in the examination of normal blood will enable the observer to recognize the invading organism with certainty.

The presence in the blood of free "pigment," probably stainable material from decomposed blood-cells or parasites, is characteristic of malaria.

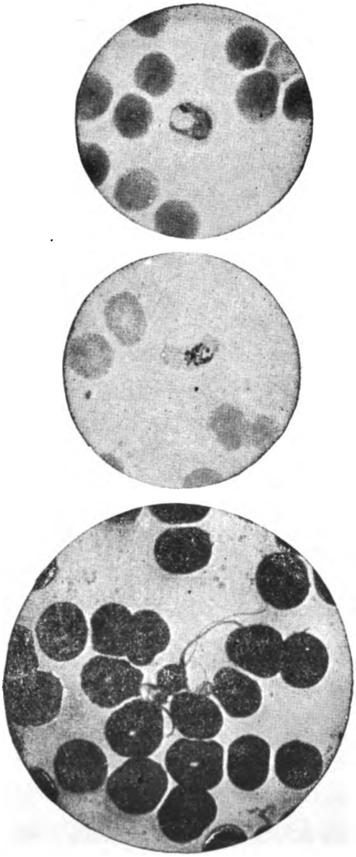

FIG. 45.—THE MALARIAL PARASITE (STAINED). (After Slater and Spitta.) 1000 diameters.

6. The Examination of Sputum for Tubercle Bacilli, and the Cultural Diagnosis of Diphtheria.—Besides the examination of blood smears for malaria the bacteriologist of a Board of Health is expected to make diagnoses of diphtheria and tuberculosis, which are of special importance on account of the necessity for prompt treatment of the former disease and on account of the difficulty sometimes experienced in recognizing the latter. Both maladies are due to the presence of bacteria, very minute rod-shaped fungi which first gain lodgment in the respiratory or alimentary passages.

In the case of consumption or pulmonary tuberculosis the bacteria are discharged in enormous numbers in the sputum and may there be detected without great difficulty. When stained with an anilin-dye the tubercle bacilli are not decolorized by dilute sulphuric acid as are most bacteria, and this property is made the basis for a simple method of differential staining. A thick smear of sputum is made on a glass slide and dried over the flame. This is stained for two minutes, heating until it steams, with Ziehl-Neelsen's carbol fuchsin (1 gram basic fuchsin in 10 cc. 95% alcohol mixed with 90 cc. of a 5% aqueous solution of phenol). The slide is then washed under the tap and immersed for two minutes in a solution of 3 parts of hydrochloric acid in 100 parts of 95% alcohol. It is then washed again and counterstained for half a minute in a 1% aqueous solution of methylene blue, washed, dried, and mounted. The slender tubercle bacilli should be stained bright red with fuchsin, while other bacteria and the leucocytes and other cell elements present are colored faintly blue.

In all work with bacteria, the $\frac{1}{12}$-inch objective must be used with a drop of cedar-oil between the lens and the cover-glass, or the slide, if no cover-glass be used. The Abbé condenser is also a necessity for lighting bacterial preparations.

The diagnosis of diphtheria is somewhat more complicated than that of tuberculosis and involves cultural as well as microscopical methods. The organism occurs at the back of the throat and material for examination is obtained by rubbing a sterile swab of cotton over the whitish patches which appear on the pharynx and fauces. This swab is then passed over a slanting surface of coagulated blood-serum upon which after incubation for six to twelve hours at the body temperature minute pearly colonies of the bacilli will appear. A portion of the growth is then removed with a sterile loop of platinum wire and spread over a clean cover-slip in a drop of water. The cover thus prepared is dried, fixed by passing three times through the flame, and stained for ten minutes with Loeffler's methylene blue (30 cc. of a saturated alcoholic solution of the dye mixed with 100 cc. of a $^1/_{10,000}$ aqueous solution of caustic potash). After washing, drying, and mounting in balsam, the specimen, in cases of diphtheria, will show the very variable rods characteristic of the diphtheria bacillus, many of them cigar-shaped or club-shaped, and often exhibiting the peculiar cross-barred appearance indicated in Fig. 46. Sometimes these organisms will be seen mixed with spherical bacteria arranged in pairs and chains, the streptococci. In negative cultures only cocci are found, as a rule, although other bacilli, more or less closely resembling the diphtheria organism, may be present.

7. The Serum Reaction in Typhoid Fever.—One of the most important practical results of the recent marvellous progress in the study of the phenomena of immunity has been a method for the clinical diagnosis of typhoid fever and certain other diseases. It has been shown that when the animal body is invaded by a parasitic micro-organism, the cells produce in some cases antitoxins which neutralize the poisons of the microbe, and in other cases anti-bodies of another type which have a specific destructive action on the parasite itself. Sometimes the defensive secretions dissolve the

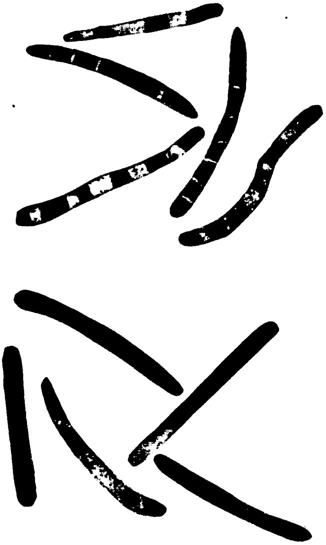

Fig. 46.—Diphtheria Bacilli (Methylene Blue). (After Wesbrook.) Diagrammatic.

foreign cells; sometimes they cause them to clump together in masses and to settle out of the fluid in which they are suspended. In the blood of an individual infected with typhoid fever, bodies of the last type, known as agglutinins, are present; and their clumping reaction is specific for that particular micro-organism. It is true that the blood of a normal individual may contain substances which agglutinate typhoid bacilli, but that of a typhoid patient has the power to such a high degree that it will produce the same effect much more rapidly and in high dilution. In making the diagnosis of a suspected typhoid case, a sample of blood is taken from the lobe of the ear in the usual manner and allowed to clot in a small test-tube so that the corpuscles are separated from the clear straw-colored serum. A drop of serum is then mixed on a slide with 40 drops of a fresh broth culture of the typhoid bacillus. The mixture is covered and examined under the $\frac{1}{12}$-inch oil-immersion objective. At first the bacilli may be seen, after careful focussing, as minute transparent rods, singly, or in pairs and short chains, moving rapidly about across the field of the microscope. If agglutinins be present, the bacteria become motionless and clump together in masses, sometimes large enough to be visible to the naked eye. If the reaction is not obtained with a $\frac{1}{40}$ dilution, another drop of serum may be added, and if this fails, again, another. The latter dilution, $\frac{1}{10}$, should clump the bacilli in fifteen minutes if the case examined be typhoid fever; with a $\frac{1}{40}$ dilution an hour's time may be needed. The test may be made quite as well with dried blood after diluting with the proper amount of water.

8. The Microscopic Examination of Pork for Trichina. —The disease known as trichinosis is like diphtheria and typhoid fever caused by a parasitic micro-organism, although the parasite belongs to a very different group. *Trichina* is a minute worm, barely visible as a speck to the naked eye, which bores its way into the muscles of swine and there encysts itself in a calcareous nodule. If such pork be eaten, imperfectly cooked, the limy cyst is dissolved; the worms emerge and reproduce, and a myriad of their progeny penetrate the tissues of the body, causing high fever and, sometimes death. If the patient recovers from this crisis, the worms encyst themselves in the muscles and produce no further serious difficulty.

In America, trichinosis is rare in man, although some 2% of swine are affected with it, because pork is more or less thoroughly cooked before eating. In Europe, however, where pork products are eaten almost raw, the danger is serious and must be met by preventing the distribution of trichinous swine flesh. Elaborate government systems of meat inspection have been instituted in many countries; in Prussia, for example, over 25,000 officials are employed for this purpose. In the United States the Department of Agriculture for some years maintained a bureau of meat inspection which examined all pork intended for foreign export and interstate commerce, employing a large corps of microscopists.

Samples of pork to be examined for *Trichina* are taken from the diaphragm or other muscles and cut in pieces about an inch by half an inch in size. One such piece is then placed between two slides of heavy glass, mounted in a frame and provided with screws, by which they can be pressed together, thus reducing the meat to a thin transparent layer. The frame bears on its under side two ridges which fit into grooves in a special corrugated stage of large size on the microscopes designed for this purpose. Under a very low objective, of 1½-inch equivalent focus, the compressed frame is then examined, being slid along until its whole width has been covered, and then shifted to the next groove, so that a new field may be examined. In this way the whole sample may be viewed in five minutes by a trained observer.

Under the microscope the pork is seen to be made up of the long cylindrical cross-striated fibres of voluntary muscle, and if *Trichina* be present the cysts will be seen here and there as spindle-shaped, whitish bodies. In sections of trichinous pork prepared after paraffin imbedding with the microtome, the worms may be seen coiled up inside the cysts, as shown in Fig. 47.

9. The Microscopy of Drinking Water.—The sanitary quality of water depends primarily upon the presence or absence of disease germs, generally introduced in sewage. The most important evidence as to its character is therefore obtained by the bacteriological and chemical analyses, which indicate with great delicacy the presence of minute traces of sewage pollution. A microscopical examination may, however, often add information of value by showing the presence of starch-grains, yeast-cells, fragments of vegetable tissue, and certain Infusoria characteristic of decomposing material; organisms peculiar to a particular pond or stream have sometimes proved of importance in the detection of admixture of its water with that from some other source. The great importance of the microscopical examination of drinking-water lies, however, in its application to the study of those organisms which produce tastes and odors in reservoirs. Such tastes and odors are in almost all cases due to the multiplication of microscopic plants and animals belonging to the Algæ and the Protozoa; and the study and control of these organisms depends upon the systematic use of the microscope.

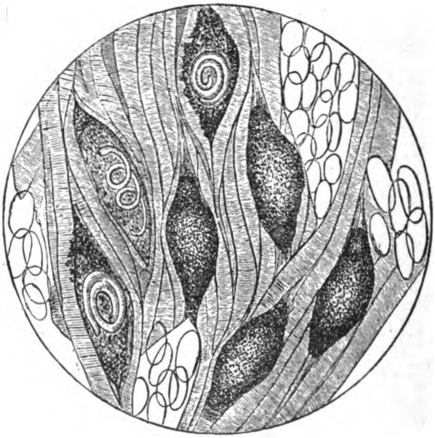

FIG. 47.—TRICHINA. (After Häger-Mez.) 50 diameters.

A considerable concentration of the sample must, of course, precede the microscopical examination of drinking-water; and this is usually accomplished by filtration through a layer of fine sand. The Sedgwick-Rafter method, in routine use by the State Board of Health and the Metropolitan Water Board of Massachusetts, the

Water Department of Brooklyn, and other sanitary authorities, is as follows: A measured quantity of water, half a liter or a liter, is filtered through perhaps a quarter of an inch of fine sand on a circle of bolting-cloth supported by a perforated rubber stopper in a tall funnel. The sand with the organisms collected on it is dropped into a test-tube, shaken up with a small volume of water, and allowed to settle for a moment. The water is then poured off and a second portion of wash-water serves to remove almost all the organisms from the sand. The two washings mixed together are then made up to some convenient volume, 10 or 15 cc., and thus one has in say 10 cc. the organisms originally present in a liter.

One cubic centimeter of the concentrate is then placed in a cell, 1 mm. deep and 1000 sq. mm. in area. A special type of ocular micrometer is used with a square ruled upon it, and the lenses and draw-tube are so adjusted that the square shall coincide with 1 sq. mm. on the stage. Ten or twenty fields in different parts of the cell are then examined and the organisms counted, when a simple calculation enables one to compute the number present in 1 cc. of the original sample.

By this method the kind and number of micro-organisms present may be easily determined, the cause of existing trouble detected, and the prospect of future difficulties predicted. The efficacy of methods of purification may be tested and the source of trouble located in the particular part of a supply where it exists.

A few of the most serious odor-producing organisms are figured in Fig. 48. At 4 is *Anabæna,* the Blue-green

Alga which has cost the city of Springfield, Mass., many thousands of dollars by the production in Ludlow Reservoir of the "pig-pen" odor. At 1 is *Asterionella*, a Diatom which caused the geranium odor in the water of Brooklyn, N. Y. The Protozoön, *Synura*, at 6 was the author of the cucumber taste in the Boston supply, and its ally, *Uroglena*, at 7, is perhaps the worst offender of all, having caused trouble in some 44 supplies in Massachusetts alone.

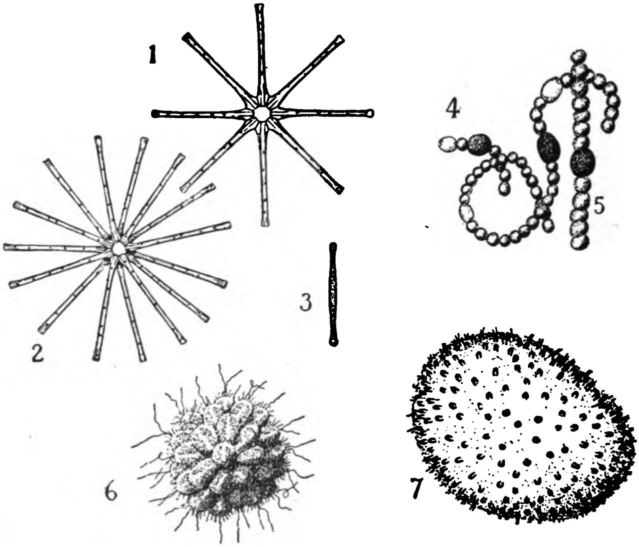

FIG. 48.—MICRO-ORGANISMS OF DRINKING-WATER. (Redrawn from Whipple.) 300 diameters.

1, 2, 3. Asterionella. 6. Synura.
4, 5. Anabæna. 7. Uroglena.

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CHAPTER X.

FORENSIC MICROSCOPY.

1. **The Microscope in Law.**—In a court of law where questions of life and death may hang upon the nature of a blood-stain or the genuineness of a document, the microscopist is often called in to study the minuter structures of disputed objects. The identification of persons by the anthropometric system of Bertillon may be supplemented to advantage by a detailed examination of the ridges upon the fingers. The detection of human blood is greatly facilitated by microscopical methods. In sexual cases the presence of spermatozoa may be easily determined under the microscope. Low powers aid in the study of bullet-wounds with respect to the distance and direction from which the shot was fired, the powder used, etc. The examination of documents, as regards the paper and writing utensil used, the presence of erasures or alterations, and the characteristics of the handwriting, is an important field for microscopy.

2. **The Examination of Blood Stains.**—Frequently the expert is asked to decide as to the character of blood-stains upon clothing, furniture, etc., stains often of minute

size and sometimes of considerable age. Two problems are involved—first, as to the presence or absence of blood in the specimen presented, and second, as to its nature, whether human or animal. The presence of blood in general may be settled pretty easily by four methods—one chemical, one microchemical, one microspectroscopical, and one purely microscopical. The chemical test consists in the application to the stain of an alcoholic solution of gum guaiacum, which yields a blue color reaction on the further addition of aerated turpentine. The microchemical test depends on the fact that hæmoglobin, the complex albuminoid substance characteristic of the red blood-corpuscles, yields on treatment with acetic acid a crystalline iron salt, hæmatin, whose triple chloride is known as hæmin. In making this test the blood, if in a dry condition, is placed on a slide and gently warmed in glacial acetic acid. This is allowed to evaporate and replaced by a 0.07% solution of sodium chloride, which in its turn is carefully evaporated without heating the slide above 60° C. Another drop of acetic acid is added and the slide is this time heated so that the liquid boils. After evaporation it is examined under the $\frac{1}{6}$-in. objective; if blood be present, crystals of hæmin (or hæmatin trichloride) will appear as minute flat rhomboids often superposed in the form of a cross. They vary from yellow to reddish brown in color and from 0.005–0.02 mm. in size. This test is a delicate one, serving to detect from 0.05–0.15 mg. of dried blood, and even very old material gives satisfactory results, as in the classic case of the blood of the murdered Kotzebue, detected sixty years after his death on the papers which lay upon his desk.

For examination by the microspectroscope, fresh blood-stains are treated with distilled water and older specimens with weak acetic or citric acid. When placed in a small glass cell and examined by the methods to be described in the next section, the characteristic spectrum of hæmoglobin or one of its derivatives will be apparent.

The direct microscopical examination of blood-stains aims simply at the detection of the red corpuscles and serves to distinguish mammalian blood from that of birds, the three tests above outlined being general for the hæmoglobin of all red-blooded animals. Dried and clotted blood must first be treated with some solvent which shall attack the albuminous coagulum and set free the blood-cells. Numerous media have been suggested, of which a 33% solution of potassium hydrate is one of the simplest, and Ranvier's solution (potassium iodide, 2 parts; saturated aqueous solution of iodin, 100 parts) is one of the best. In any case a bit of clot or the scraping from a stain is covered with the solvent and placed in a hollow slide under the microscope. Sometimes after a few minutes, sometimes only after days, the opaque mass becomes clearer and the individual cells appear as circular, non-nucleated discs 7.5 μ in diameter. Under most conditions the discs appear somewhat biconcave, with a thickened ring about the edge.

In the original process of drying or in the treatment with a solvent, the blood-cells may be so distorted that their recognition is not easy. In water, for example, the corpuscles swell up to a spherical form. The commonest objects which may be mistaken for blood-cells are the spores of certain plants, and medico-legal literature contains ludicrous instances of erroneous expert opinions based on such findings. With a good preparation carefully compared with specimens of fresh blood, such errors can easily be avoided; and, since the corpuscles of birds are of oval shape and show a distinct nucleus, blood-stains of avian origin may be excluded. The blood of other mammals can be differentiated from that of man only by the size of the cells, and the differences are so slight that a certain result can seldom be reached. The blood of the goat has cells less than 4.5 μ in diameter, while the corpuscles of the horse and cow and pig are under 6 μ; but the corpuscles of the dog, rabbit, mouse, cat, and other domestic animals very closely resemble those of human blood. Such differences of one or two micromillimeters cannot be relied upon, since even in the fresh blood of a living animal some variation occurs and distorted dried specimens are much more dubious. Dr. E. L. Walker has pointed out that the white blood-cells of different mammals differ much more widely than do the red corpuscles; and the study of these bodies may furnish valuable aid to the microscopist.

The crucial test for blood-stains, and the only method by which the blood of man may be definitely distinguished from that of other mammals, depends upon a specific biologic reaction similar to that which is used in the serum test for typhoid fever. If human blood-serum be injected into the lymph spaces of a rabbit, there appears in the blood of the rabbit a substance which precipitates certain albuminoids in the blood of man; and this reaction serves to distinguish human blood from that of the domestic animals with certainty. Only with the corpuscles of apes and monkeys does the serum of the immunized rabbit react in a similar manner. This test, which ordinarily bears the name of Bordet, who first suggested it, is not applied under the microscope, but in small tubes in which the precipitation is detected macroscopically.

3. **The Microspectroscope and Its Use.**—In testing blood-stains, and in many other fields of microbiology and microchemistry, the spectroscope may furnish aid. As ordinarily used, the instrument consists of a tube fitting on the eyepiece of a microscope and containing, as shown in Fig. 49, a triangular prism of flint glass which disperses white light into its constituent colors and two prisms of crown glass set in the opposite direction, which serve to counteract the refraction produced by the first prism. The principle is the same as that of the achromatic objective, but whereas in the latter the angles are arranged to produce a net refraction with neutralized dispersion, the prisms of the spectroscope yield a net dispersion with practically no refraction.

The special type of eyepiece used with the microspectroscope contains a diaphragm which only allows a longitudinal slit of light to pass, and the width of the slit is regulated by a set screw, being cut down for use to half a millimeter or less. When the detachable prism tube is placed in position and the field well lighted, a spectrum should be seen lying with its colored bands at right angles to the slit in the ocular. If the spectrum lines are oblique, the prism tube must be rotated until it bears the proper relation to the slit.

Reference to Fig. 49 will show also a lateral tube connected with the spectroscope, into which rays of light from outside are projected by a mirror, and a prism by which these rays are reflected upward. This is used when it is desirable to compare the spectrum of sunlight with that of some object upon the stage of the microscope. When properly adjusted the two spectra, produced respectively by light passing up through the microscope and by that reflected through the side tube, will appear to lie side by side.

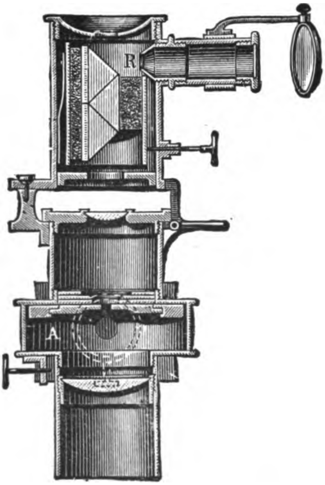

FIG. 49.—THE MICROSPECTROSCOPE. (Bausch and Lomb.)

The sensation of light is produced by the ether vibrations, whose length lies between $\frac{271}{10,000,000}$ and $\frac{155}{10,000,000}$ of an inch. When any solid body is gradually heated it becomes luminous, first emitting the longer red rays at a rate of 458 million of millions per second and later the rays of other colors as well, culminating with the violet rays, whose rate is 727 million of millions. At this point the body has reached a white heat, since rays of all colors are given off. If now the white light from such an incandescent body be passed through the spectroscope, it is broken up, through the different refrangibility of the rays of different amplitude, into a continuous spectrum in which all the rainbow colors appear merging into each other. An incandescent gas, on the other hand, produces a line spectrum, most of the field being dark, with here and there narrow bright lines whose number and position are characteristic of the particular substance.

The color of objects is due to their property of transmitting or reflecting rays of a certain amplitude. If white light be passed through certain solids, liquids, or gases below their point of incandescence, and then through the spectroscope, the presence of black bands crossing the spectrum shows that light of certain definite wave-lengths has been removed. A gas cuts out the same light-waves which it itself produces at a higher temperature.

The spectrum produced by sunlight is a continuous one, like that produced by other incandescent solids, crossed here and there by dark bands, the Frauenhofer lines, which are due to the presence of the cooling vapors of metallic elements in the surrounding atmosphere, each of which absorbs the light corresponding to its own bright line spectrum. The Frauenhofer lines may be considered the shadows of the spectra of the metals in the outer atmosphere of the sun.

Obviously artificial light should be used for the microspectroscope, since the Frauenhofer lines would introduce a confusing element. When various solid and liquid substances are placed on the stage of the microscope, absorption spectra of the same general type as that of sunlight are produced, although the dark bands are wider and less sharply defined, this being more and more the case as the thickness of the body is increased. It is therefore desirable to examine layers of varying depth, and this may be done by means of a cell made by cementing to a slide a piece of heavy glass tubing cut off obliquely so that it shall be 5 mm. deep on one side and 0.5 mm. deep on the other.

The application of the microspectroscope to the examination of various organic bodies has not yet been developed to anything like its possible limits. It has yielded interesting results in the study of certain animal and vegetable coloring-matters; but its chief use has so far been in the detection of blood-stains, since the red coloring-matter of blood produces characteristic spectra. Suspected blood-stains which are to be thus examined may be treated with distilled water until a reddish-brown solution is produced, when it is apparent that hæmoglobin has gone into solution. The liquid thus obtained, when placed in a cell, covered and examined with the low objective, shows the spectrum of oxyhæmoglobin with dark bands in the yellow and green, shown in Fig. 50 at *A*. If a drop of ammonium sulphide be added, the two bands will fuse into one, giving the spectrum of reduced hæmoglobin, shown at *B*.

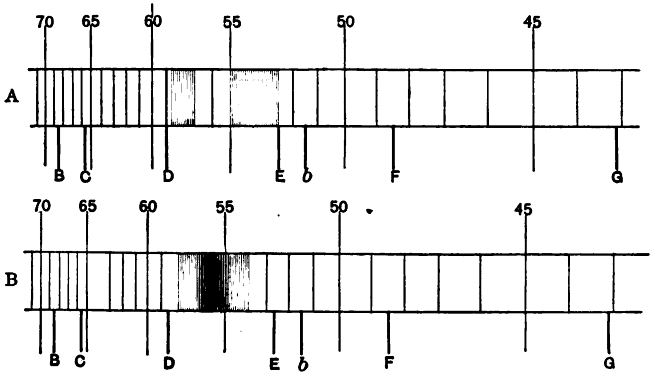

Fig. 50.—Spectra of Oxyhæmoglobin and reduced Hæmoglobin. (After Howell.)

The addition of a little citric acid to a solution of oxyhæmoglobin produces the spectrum of acid hæmatin, with a broad band in the red, a narrow one in the green, and a very faint band in the blue. In old blood-stains the oxyhæmoglobin has been changed to hæmatin, and since this substance is insoluble in water, no coloration of the liquid appears when the stain is treated as above. In such a case a little acetic acid must be tried; this dissolves hæmatin, and the solution at once produces the acid hæmatin spectrum. On adding ammonia, the broad band in the red disappears. Blood-stains in which the hæmoglobin is only partially changed to hæmatin (methæmoglobin) may show a spectrum with the combined bands of both. The spectroscopic test is, of course, given by the blood of any red-blooded animal. It is extremely delicate and has yielded positive results with stains over a hundred years old.

4. **The Study of Finger-prints.**—The identification of individuals, so important not only in criminology but in a multitude of civil and criminal cases, has been greatly facilitated by the anthropometric system of M. Bertillon. This rests upon the principle that variations in the proportions of the body, when a number of separate features are measured, are so characteristic that a definite formula may be obtained from them, which shall differentiate each individual from any other. Commonly the length and breadth of the head and the length of the left middle finger, the left forearm, and the left foot form a primary basis for classification to which other general characteristics and special individual peculiarities may be added *ad infinitum.*

The use of the finer details of the structure of the skin to supplement the Bertillon system was first suggested by the distinguished English biologist, Francis Galton, though Sir William Herschel had previously used finger-prints as a means of personal identification. Recently Professor H. H. Wilder's investigations have shown the great importance of these structures in the study of heredity. If the bulb below the tip of the finger be pressed first upon a metal surface covered with a thin film of printer's ink, and then upon a paper or card, a permanent record will be obtained of the arrangement of the ridges and furrows characteristic of that digit, a record necessarily free from the error which may attend ordinary anthropometric measurements. Galton has shown that the pattern of the skin persists unchanged through life and is unaltered in its essentials by cuts, burns, or any ordinary accidents. The variations are so great that he calculated the chances of identity between two single prints to be only one in sixty-four billion. When ten digits are compared, identification is absolute.

Such records, in order to be of any practical service, must be readily arranged and classified; this Galton has done so successfully that, out of a "finger-print directory" of 2632 persons, any pattern could be located in three minutes. In the first place, the patterns of the skin may be grouped under three heads. In all cases the papillary ridges run across the fingers in the vicinity of the third joint, and at the tip they follow the curve of the nail in a rounded arch. Sometimes the ridges between follow the outer ones in a more or less even arch of lessening convexity; this is the arch type (1, Fig. 51). Sometimes the intermediate ridges form a loop running from one side inward to the center of the bulb and then doubling back again. Obviously at the opposite side

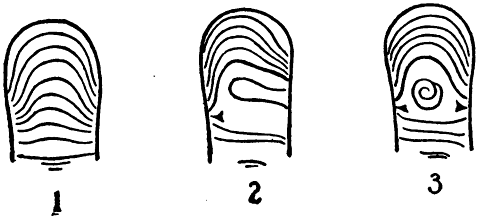

FIG. 51.—FINGER-PRINT PATTERNS. (Redrawn after Galton.)

of the finger the outer ridges, the loop, and the basal ridges will cut off between them a triangle, known as the delta (2, Fig. 51); this is the loop type. Finally, the ridges on the bulb may be so twisted as to form a complete circle cutting off two deltas, one on either side; this is the whorl type (3, Fig. 51).

A classification of the ten digits according to their arches, loops, and whorls serves for a primary division of finger-print cards into a number of general classes. Since, however, certain combinations of these digital formulæ are much more common than others, further subdivision must be made; and this may be accomplished by studying the minuter structures of the finger-print, and particularly by counting the ridges in the loops of those digits which show that structure. It is here that the microscope comes into play, since such details cannot well be made out with the naked eye. A magnification of only ten or twenty diameters is, however, generally desirable; hence the instrument used must be either a simple microscope, preferably mounted on a stand, or a compound microscope of very low power and very wide field.

5. The Examination of Documents.—In the study of disputed documents the microscope may be of considerable service to the legal expert. The material of which paper is made, its texture and sizing, and its water-marks should first be noted, the character of the writing instrument and the grooves it cuts upon the paper, if a steel pen, being possibly significant. Inks may be tested by various chemical methods; but much may be learned under the microscope from an observation of the color and metallic luster of the deposit formed by inks containing gums and of the solid particles of coloring-matter always present. Even the changes of consistency in the same bottle on standing may be detected by this method.

Direct evidence of forgery in the shape of alterations, erasures, or interlineations is often furnished by examination with a hand lens or a low-power compound microscope. Erasures remove the sizing and loading material of the paper and leave loose ends of teased-out fibres in which the ink of later writings runs freely. Marks of preliminary tracings are sometimes apparent in cases of elaborate forgery. When two lines cross each other it may be of importance to determine which was made first; and here, too, the microscope is of service, since the upper line often shows a widening, due to capillarity, on entering the lower line, and a narrowing on leaving it. The continuity of the pen furrows of the upper line may also be apparent. Obliquely viewing the point of intersection with a hand lens, along the two lines successively, helps to make it clear which one was superposed.

Proceeding to the handwriting itself, the general spacing of the lines, words, and letters should first be noted. The pen pressure, the shading, the general symmetry of words and letters, and the firmness of individual lines are all significant. Temperament manifests itself in haste or caution, in energy or reserve. Lack of facility, physical feebleness, and the labored attempt to imitate, alike produce tremors which may easily be distinguished from each other.

All these obvious peculiarities may be imitated by a skilful hand, and it is only more minute characteristics which serve for the certain identification of handwriting.

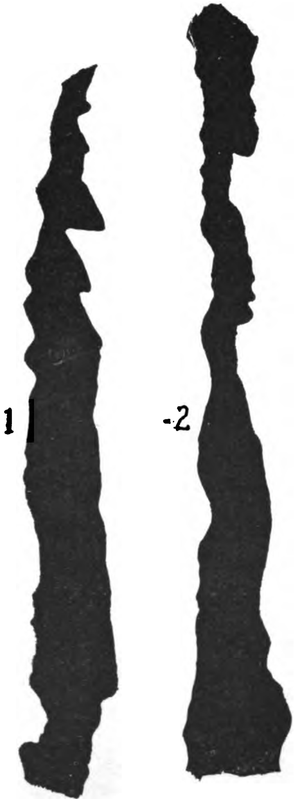

FIG. 52.—MINUTE CHARACTERISTICS OF HANDWRITING LINES. (After Frazer.)

The latter are studied by two methods, the statistical and the microscopical. First, the measurements of the distance between certain letters in a given word, of the ratio of heights to breadths in certain letters, and of the angular slope of certain long strokes, when averaged, yield values from which the deviations of the writer are rarely great. Composite photographs of signatures, etc., are important aids in work of this sort. In the second place, more minute differences exist which can be detected only under the compound microscope. These are of three magnitudes. First, any long lines show certain variations of direction whose number and nature vary with the writer. Second, much finer fluctuations occur, visible only under the compound microscope, in the shape of vertical deviations from side to side and changes in width due to periodic changes in the pressure of the pen-point. Third, on one or both margins of the line are still more minute lateral serrations. These vertical and lateral variations are both illustrated in Fig. 52. They are, of course, influenced more or less by paper and writing utensils and by the physical and mental condition of the writer; yet the size, position, and number of the waves, swellings, and notches are so related to the nervous organization of the writer, and differ so markedly with different individuals, as to yield important aid in the identification of handwriting.

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CHAPTER XI.

MICROCHEMISTRY.

1. The Application of Microchemical Analysis. — In few fields have the possibilities of the microscope been less fully realized than in pure and applied chemistry. A science which deals with the character and behavior of chemical substances should surely take into account the characteristic crystals of which, under certain conditions, many such substances are composed. Yet chemists trained to study gross color reactions and precipitates have too often regarded the microscope as a strange and impracticable instrument. Systems of recondite and elaborate secondary tests have been built up, while the direct study of fundamental physical characters has been neglected. Although the toxicologist and the medical microscopist have used microchemical tests with such good results that in those special fields they are of importance, in general the adoption of these methods by chemists is slow; and only the development of petrography, the microscopic study of rock sections, has at last, during very recent years, called general attention to their importance.

The study of microchemistry is by no means limited to the examination of crystalline forms alone, as might be supposed from a perusal of its earliest exposition, published by Harting in 1866. Lehmann, Behrens, and others have since shown that many dynamic properties, melting and volatilization for example, as well as the typical chemical reactions, may often be observed to great advantage under the microscope. The use of minute quantities of material (one-tenth of a milligram often sufficing), the rapidity with which results may be obtained, and the simplicity and compactness of the apparatus needed are general advantages of the microchemical method, which apply in all manner of analyses. On the whole, few chemists who take the trouble to familiarize themselves with the use of the microscope will fail to find it at times a valuable aid in their work. In the study of certain closely related organic compounds it furnishes at once information which can otherwise be attained, if at all, only by the expense of a vast amount of labor.

2. **The Study of Typical Crystals.**—It will be well for the student of microchemistry to familiarize himself first with the most important characteristics used in the identification of crystals, by the examination of certain typical forms. The actual process of deposition should be studied and those differences observed which occur in the formation of crystals under different conditions, as, for instance, from alcoholic and aqueous solutions, and by slow spontaneous drying on the one hand and rapid evaporation by heat on the other. Crystals formed in a mother-liquor of the same composition show their characteristics with special distinctness.

The linear dimensions and the relative proportions of the various faces of a crystal vary widely with the supply of material in the solution from which they were deposited. The interfacial angles between corresponding faces and the facial angles between corresponding edges are, however, always constant for a given substance. This law, known as the Law of Steno, forms the basis for determinative crystallography, and makes it possible to distinguish the crystals of such compounds as are not isomorphous.

Besides the general crystalline form, there are numerous special points to be noted in the study of a crystal. Its habitus (the size and proportions due to the conditions attending its deposition) should be noticed, as well as its color and the approximate refractive index, indicated by the definition of the edges either in air or in some denser mounting medium. The effect upon polarized light, which will be discussed in Chapter XII, is often of great importance. Various peculiar phenomena may appear, such as hemimorphism (differences in the opposite ends of a crystal), twinning (the production of double crystals), skeleton crystals (parallel or symmetrical aggregates of smaller crystals), trichites (hair-like crystals, often more or less twisted), and sphærulites (radially fibrous spherical bodies). Sodium chloride crystallized rapidly from a solution thickened with mucilage exhibits beautiful skeleton crystals; and long, curved trichites appear in a mixture of the chlorides of chromium and mercury.

The change in crystalline form of certain compound salts furnishes an interesting subject for study under the microscope. If ferrous chloride is allowed to crystallize from a warm, aqueous solution, large, tabular, monosymmetric (rhombic) crystals form. If, now, the slide be quickly heated by the application of a small flame, the large plates fall to pieces, and minute crystals of the anhydrous salt appear in their place (Fig. 53). On cooling, the small crystals take up water and run together in their original form.

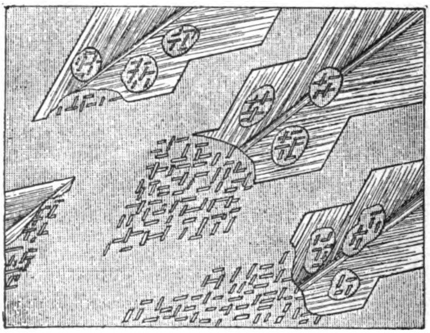

FIG. 53.—CRYSTALS OF HYDROUS AND ANHYDROUS FERROUS CHLORIDE. (After Lehmann.)

Behrens and other authors give a large number of specific microchemical tests, some of which the student should make in order to gain practice in the recognition of typical crystalline forms. Aluminium, for example, may be easily detected in a solution by evaporating with a small drop of sulphuric acid, dissolving the residue in water, and adding a grain of cæsium chloride. Large, colorless, isometric octahedra of cæsium alum are produced, or, if more than 1% of aluminium sulphate be present, rectangular dendrites sprout from the cæsium salt (Fig. 54).

Calcium is best tested for by precipitation with sulphuric acid, which in concentrated solution throws out short orthorhombic crystals of anhydrous calcium sulphate. From dilute acid solutions slender monoclinic prisms of the compound ($CaSO_4 + 2H_2O$) crystallize out (Fig. 55), showing numerous double twins, as at *C*, and in presence of strong acids, masses of minute needle-like crystals, as at *A*.

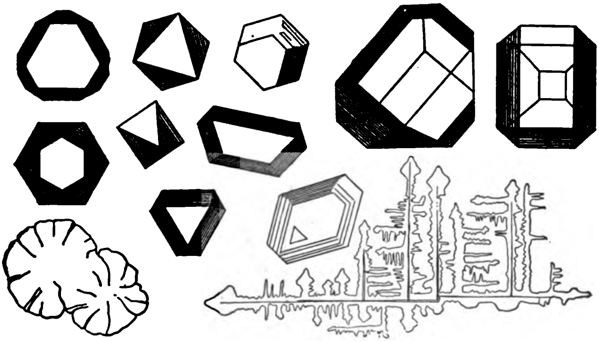

FIG. 54.—CRYSTALS OF CÆSIUM ALUM. (After Lehmann.)

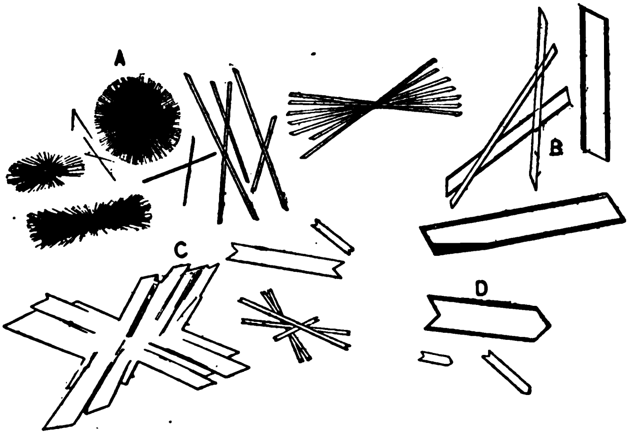

FIG. 55.—CRYSTALS OF CALCIUM SULPHATE. (After Lehmann.)

3. The Detection of Quinin.—It has been noted that one of the earliest and most profitable applications of microchemistry has been made in the study of drugs and poisons; and the toxicologist depends to a great extent upon various specific microchemical tests. Herapath's method for the detection of quinin, applied particularly to the examination of the urine of patients under quinin treatment, may be studied as an example. It depends on the formation of characteristic crystals of the iodosulphate of quinin on the addition of tincture of iodin to an alcoholic solution of the drug. In practice, the urine to be analyzed is neutralized and shaken out with ether. The ethereal solution is evaporated and the residue dissolved in a mixture of 12 cc. of glacial acetic acid and 4 cc. of 95% alcohol, to which 7 drops of dilute sulphuric acid (1 gram of strong sulphuric acid with 9 grams of water) have been added. A very minute drop of tincture of iodin added to this solution first produces, if quinin be present, a cinnamon-yellow spot, due to the reaction of the iodin and quinin. Next the alcohol separates in little drops, driving the fluid away from the center of the preparation. Finally, the acid liquid flows back again, and thin, greenish plates of the iodosulphate appear, often arranged in beautiful rosette forms. The crystals produce strong polarizing effects, and turn brownish red when heated.

3. The Separation of Related Organic Substances.—The most important aid which the microscope can offer to the chemist lies in the domain of organic analysis. Lehmann even goes so far as to say that "Crystallography is to the organic laboratory what spectrum analysis is to the inorganic." When it is necessary to separate two closely related isomers, to determine the identity of two substances prepared by different methods, to distinguish differences of elementary composition from those due to allotropism, or to detect minute admixtures of an impurity, it frequently occurs that purely chemical methods are tedious and complex, while microscopical examination furnishes a rapid and easy solution. In general the method of study consists in allowing the substance in question to crystallize in contact with a body of known composition, with which it is to be compared. This may be done in three ways—by adding to one substance, in a melted condition or in solution, a crystal of the other, by preparing a mixture of both in a liquid condition and allowing the mixture to cool, and best, perhaps, by crystallizing from films of the two substances brought just in contact with each other.

When solid substances are to be compared, a small portion of one is placed under a cover-slip and heated till it melts, the amount of material being so small as not to reach the edge of the cover. A grain of the second substance is then placed just outside the cover-slip, and in turn melted so as to flow under and join the first along a line which can be observed under the microscope. (Incidentally it may be noted that by heating two bodies simultaneously under the microscope a good measure is obtained of slight differences in melting-points. Thus the similar crystals of dinitrobenzol and dinitrotoluol may be easily distinguished.) As two substances cool and recrystallize, the phenomena along the contact zone are diverse and striking. In some cases the mixture of the two materials remains liquid longer han the pure substances on each side, forming a clear area in which, later, an amorphous precipitate is deposited. The chlorides of iodin and silver exhibit this case to perfection (Fig. 56). Or a crystalline precipitate different from either of the pure substances may form in the contact zone, as with silver and potassium iodides. Or the crystals may grow across the line unchanged, forming a single homogeneous mass. This occurs with identical bodies and with isomorphous substances like dibrombenzol and dichlorbenzol. Or the crystals may grow across the contact zone, but with spaces between them, due to the presence of impurities in one or other of the substances. Usually the habitus of the crystals in the impure substance will be somewhat changed. Pure dinitrotoluol and the same body containing paranitrotoluol exhibit this phenomenon. These four cases by no means exhaust the possible conditions of the contact zone, but they serve as types of some of the more striking ones,

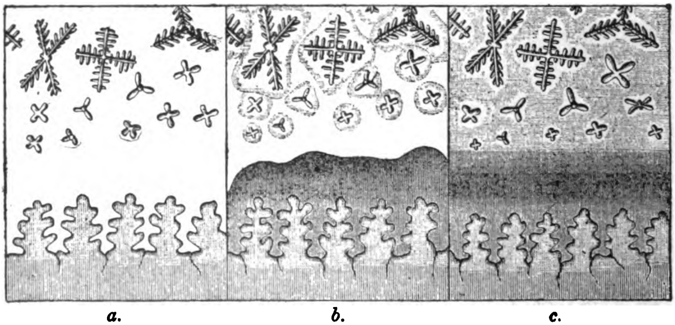

FIG. 56.—CONTACT ZONE BETWEEN CHLORIDES OF SILVER AND IODIN. (After Lehmann.)

and may suggest how the microscope serves to test the identity and the purity of organic bodies.

5. The Methods of Systematic Microchemical Analysis.—We have seen that microchemistry is a much wider term than crystallography, and covers the study of change, of state and of chemical reactions as well as crystalline form. In this broad sense, specific microchemical tests have been suggested by Behrens and others for the detection of the elements and the various acid radicles. Recently Hinrichs has prepared a complete outline for qualitative analysis under the microscope. Whether such a scheme will ever come into general use is doubtful, although it promises results with a minimum of time and material; but the study of two typical basic and acid groups may prove suggestive to the student.

A microburner yielding a flame not more than a quarter of an inch in height must be provided for melting the substances examined, and it is convenient to have the lamp so small that it may be placed under the stage of the microscope if desired. For ordinary work microscopic slides are used, a drop of the substance to be tested being placed thereon with a fine glass stirring-rod. The reagent is added in minute quantity and is made to mix with the first by means of a dry stirring-rod. For sublimation tests, two 1-inch watch-glasses are used, one inverted over the other, with the substance to be examined in the lower, and the appropriate test reagent borne on the concave surface of the upper, glass.

6. The Determination of the Metallic Elements.—According to the scheme prepared by Hinrichs, solutions of the metallic elements are first tested by the addition of a fragment of metallic zinc. If metallic crystals form, either silver, copper, bismuth, lead, tin, cadmium, or thallium is present. If a blackish coating appears upon the zinc, gold, platinum, iridium, palladium, or mercury is indicated. Should neither phenomenon follow, the other groups of elements may be tested for by the addition of nitric acid, magnesium ribbon, ammonium chloride, and magnesium and ammonium-phosphate solution; we are here, however, concerned only with the first group of metals, as typical of the general method of analysis.

If the solution contains one of the metals precipitated by zinc, drops of it must be tested with several reagents, each of which points out certain of the elements. Thus a grain of potassium chloride is added, and with silver, lead, and thallium a characteristic reaction is obtained. Silver salts produce an immediate white curdy precipitate of silver chloride, lead salts, crystals of lead chloride, and thallium compounds, very minute characteristic crystals of thallium chloride. In each case confirmatory tests must be applied. With silver, for example, the amorphous precipitate should be first separated by pouring off the liquid and then dissolved in a drop of ammonium hydrate. The colorless solution thus obtained on warming and then cooling yields large cubical crystals of silver chloride. If silver be present, a drop of the original solution, warmed and made faintly acid with nitric acid, produces on the addition of potassium bichromate large dark-red hexagonal plates and prisms of silver bichromate, soluble both in nitric acid and ammonia. A grain of metallic lead added to a solution of silver salts causes the separation of metallic silver in the form of dendritic skeleton crystals.

The other elements are identified by similar specific reactions. Copper is at once indicated by the color of the reduced metal and the solution. Bismuth is detected by the large hexagonal plates of bismuth sulphate formed by warming with sulphuric acid and again cooling. Tin solutions, with an acid reaction, on the addition of sodium iodide, produce characteristic crystals of sodium iodostannites and iodostannates. Cadmium forms a white precipitate with ammonium hydrate.

7. The Determination of the Acid Radicles. — In studying the acid radicles in an unknown solution, it is first necessary to determine the presence or absence of organic acids. This may be accomplished by mixing a drop with a minute drop of a saturated solution of potassium permanganate and adding concentrated sulphuric acid. In the presence of organic acids, nitrites, sulphites, and hyposulphites, the pink solution is decolorized. The last three classes of compounds produce decolorization when acetic acid is substituted for sulphuric acid, while the organic bodies do not.

If organic acids be absent, a drop of the solution is tested for volatilization with acetic acid and sulphuric acid successively. A drop of silver nitrate placed on the under side of the upper watch-glass serves to absorb the vapors and record their presence by precipitation. The first group of acids decomposed by acetic acid includes the nitrites, cyanides, carbonates, sulphites, hyposulphites, sulphides, and hypochlorites. The second group, unaffected by acetic acid but volatilized by sulphuric acid, includes nitrates, acetates, chlorates, chlorides, bromides, iodides, cyanoferrates, cyaniferrates, and borates. The third group of non-volatile acids comprises the sulphates, phosphates, arsenates, and arsenites (colorless solutions), the permanganates, bichromates, and chromates (colored solutions), and the silicates, molybdates, and tungstates (precipitates).

In each case specific confirmatory tests are made for each of the acids indicated. In the two first groups the volatilization is repeated twice, once with silver nitrate and once with lead acetate on the upper watch-glass. Nitrates yield negative results with the first and octahedral crystals of lead nitrate with the second. Acetates show the long white prisms with rhombic ends characteristic of silver acetate, and no reaction with the second reagent. Chlorides are characterized by thick rhombic prisms of lead chloride and minute cubical crystals of silver chloride. In each case comparison with known substances makes it possible to identify the compound present without serious difficulty.

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CHAPTER XII.

PETROGRAPHY AND METALLOGRAPHY.

1. **The Study of Rock Sections.**—The determination of minerals can be more satisfactorily carried out by the microscopic study of thin sections than by any other method, and the microscope occupies almost as important a position in the geological laboratory as in that of the biologist. Frequently optical characteristics are the only ones which can be relied on for the identification of a given mineral, and almost always they are more easily made out than the chemical composition. The presence and character of minute impurities are detected with facility under the microscope, and much information may be obtained with regard to the physical conditions which attended the genesis of rock formations. The study of the minute structure of fossils forms a subordinate but attractive branch of the general subject.

For examination with the microscope, it is necessary to prepare sections of rocks so thin as to be transparent; and this is a task of some little difficulty. When the necessary apparatus is at hand, slices are first cut with a lathe, using for a saw a thin disc of iron, copper, or sheet tin charged along the edge with diamond-dust or fine emery. It is, however, generally possible to detach with a hammer a chip of suitable form without using a special section-cutter. The chip or section is then ground down on an emery-wheel or by hand, first on an iron or copper plate covered with No. 120 emery and water, then on a glass plate with flour of emery. The slice thus prepared is cemented to a piece of glass about one inch square. Canada balsam may be used as a cement, the specimen being pressed down into the warm balsam, with care that bubbles are not included, the slide being then heated till the balsam becomes hard and firm. After cementing, the free surface of the section is ground down until the required degree of transparency has been attained, the glass serving as a holder and support. A good section should not be over 0.05 mm. in thickness.

Certain opaque minerals are observed by reflected light, and in many cases it is convenient to examine mineral powders directly. They may be mounted in water for the study of the form of the particles, and in glycerin or some similar highly refractive substance for internal structure.

The color and form of the crystalline or amorphous constituents of a rock are first observed under the microscope. Appearances of high or low relief indicate differences of refraction. The presence of cleavage lines and the occurrence of inclusions of gaseous, liquid, or glassy character should be noted. The types of crystals present may be made out, care being taken to interpret correctly the peculiar appearances due to the plane in which the section studied may happen to lie. Even the angle of the crystals can often be measured with a microscope provided with a revolving circular stage, such as should be used for all petrographical work. The intersection of cross-hairs placed in the eyepiece is adjusted to coincide with the vertex of an angle, and one of the cross-hairs is made to coincide with one side of the crystal; the stage is then rotated until the adjacent side lies along the cross-hair and the angle of rotation measured on the graduated edge of the stage. Incomplete crystals without definite outlines, corroded crystals affected by the molten magma, broken or strained crystals, and various types of incipient crystals may be made out. Finally no petrographic examination is complete without the study of polarizing-properties. Before going further, therefore, it is necessary to describe the micropolariscope and its application.

2. **The Micropolariscope.** — Ordinary light is made up of vibrations of ether in all possible directions which lie in a plane at right angles to the direction of transmission of the light-ray. The path of an individual particle of ether in ordinary light would therefore be constantly changing. Certain substances offer resistance to the passage of such a light-ray, and confine its vibrations to two planes at right angles to each other. Light which, like that of the two resultant rays, vibrates only in one plane, is known as plane polarized light. The two rays produced, with vibrations in planes at right angles to each other, obey different laws of refraction. Thick layers of doubly refracting bodies like calcite cause so wide a separation of the two rays as to produce two

images of an object viewed through them (double refraction).

The polariscope is an instrument for detecting the polarizing power or double refraction of bodies, and consists, in essence, of a pair of Nicol prisms, each of which is made by cutting a rhomb of calcite diagonally and cementing the halves together again, with a layer of Canada balsam between them. Through such a prism (Fig. 57) one of the rays of plane polarized light (*EFGH*), known as the extraordinary ray, passes unchanged, while the other (*EFKL*), the ordinary ray, is so strongly refracted in the first half of the prism that it meets the layer of balsam at such an angle as to be totally reflected from the balsam surface, and is thus removed. A Nicol prism is, then, a device for removing

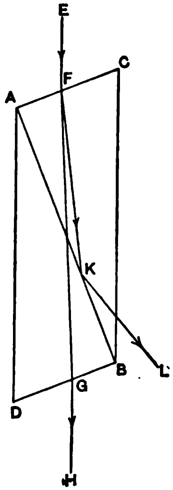

FIG. 57.—DIAGRAM OF A NICOL PRISM. (After Clark.)

the ordinary ray from plane polarized light and allowing only the extraordinary ray to pass.

For studying polarization phenomena, the microscope is provided with two Nicol prisms, properly mounted, one placed below the stage and known as the polarizer, the other inserted, as a rule, in the tube of the microscope above the objective, and known as the analyzer. One or both of the Nicols and the stage of the microscope are arranged to be rotated at will. The polarizer breaks up the light from the mirror into two rays of plane polarized light, and of these suppresses the ordinary ray. The remaining extraordinary ray passes up through the stage and objective of the microscope to the analyzer. When the analyzer occupies two positions relative to the polarizer, one in which the oblique cut surfaces of the two are parallel, and another at 180° from this, it is as if they were continuous, and the ray of plane polarized light passes freely. At the intermediate points, 90° from the parallel position, no light passes at all, since the extraordinary ray bears to the second prism the same relation which the ordinary ray does to the first, and is therefore suppressed. At intermediate points the ray is broken up into two components, with vibrations at right angles to each other, and one of these components passes the analyzer, producing a sort of twilight, the intensity of which increases as the parallel position is approached.

If, now, the polarizer and analyzer be crossed, placed, that is, at 90° from the parallel position, no light passes and the field of the microscope remains dark. If crystals of sodium chloride or some other salt crystallizing in the isometric system be mounted on a slide and placed on the stage of the microscope, the stage still remains dark. Conditions are unchanged, since amorphous substances like glass and crystals of the isometric system produce no polarizing effects. When calcium-carbonate crystals, or those of any other substance of the last five systems, are examined with crossed Nicols, the crystals become visible as shining bodies on a black ground. They exercise a polarizing action of their own upon the plane polarized light which passes through them, breaking it up into two rays polarized at right angles to each other and not coincident with the plane in which the light is suppressed by the analyzer. Thus, when viewed with crossed Nicols, polarizing or anisotropic bodies become luminous, while non-polarizing or isotropic bodies do not.

One other phenomenon must be noted in the most rudimentary account of the polariscope, the production of color effects by thin plates of anisotropic substances. If a plate of mica be placed upon the stage of the microscope, the entering ray of plane polarized light, which we may call A, is resolved into two rays with vibrations at right angles to each other, A' and B'. Of these, the ordinary ray B' at right angles to A and A' is most refracted, passes through a greater distance in the mica than does the extraordinary ray, and is retarded by an amount depending on the thickness of the plate and the strength of double refraction of the substance. In the analyzer each of the two rays A' and B' is split up again into two components at right angles to each other, $A'a$ and $A'b$, *B′a* and *B′b*. *A′a* and *B′a* are parallel to the plane of the original illuminating ray *A*, and are suppressed. *A′b* and *B′b* lie in the plane at right angles to this and pass freely. As they unite, however, their waves are, in different phases, due to the retardation of *B′* in passing through the mica plate, and if the amount of this retardation be right, interference phenomena will be set up and very beautiful color effects produced. The particular color varies with the thickness of the plate examined; but at the two opposite positions of the analyzer at which most light passes, the colors which appear are complementary. Using the notation adopted above for illustration, in one case *A′a* and *B′a*, in the other, *A′b* and *B′b*, pass; and, since the sum of all these four rays originally produced white light, it is obvious that the complementary relation must exist.

3. **The Identification of Minerals.**—Besides the general microscopical appearance of crystals noted above, it is possible with the polariscope to study one character of perhaps more practical importance than any other, the optical structure as indicated by its effect on polarized light. We have seen that crystals of the first or isometric system are isotropic. All other crystals are anisotropic, but in those belonging to the tetragonal and hexagonal systems there is one axis about which the structure of the crystals is homogeneous, and along this axis light passes unaffected. Such crystals are called uniaxial, while crystals of the orthorhombic, monoclinic, and triclinic systems possess two such axes, and are called biaxial.

Examination with crossed Nicols makes it possible at once to distinguish isometric crystals from those of other systems. Good crystals to compare in this respect are garnet and gypsum, the former being isotropic, the latter anisotropic. The same minerals furnish an instructive contrast in refractive index, the former showing high and the latter low relief.

Tetragonal, hexagonal, and orthorhombic crystals may be separated from those of the monoclinic and triclinic types by the fact that in them the directions of vibration (sometimes called axes of elasticity) are parallel to the crystal axes of the crystal. The position of these vibration directions can be determined by noting the position in which the crystal becomes dark (position of extinction) with crossed Nicols, for it is then that the vibration directions coincide with the planes of the Nicols. A cross-wire placed in the eyepiece so as to coincide with the plane of vibration of the analyzer under these conditions coincides also with the axis of elasticity of the crystal. By now removing the analyzer, the cleavage lines and boundaries of the crystal may be seen and the stage rotated so that the cross-wire corresponds with them. The angle of rotation required to produce this effect as measured on the graduated edge of the stage, if 0° or 90°, on all the crystals examined, indicates a tetragonal, hexagonal, or orthorhombic crystal. Such substances are said to show no extinction angles or to be symmetrical. Quartz (hexagonal) illustrates this condition, while in Gypsum (monoclinic) all planes but one show large extinction angles. This, like other monoclinic minerals, exhibits in one plane phenomena similar to those characteristic of hexagonal crystals, and shows no extinction angle.

When certain sections of anisotropic crystals are examined with convergent polarized light obtained by a converging lens fitted over the polarizer, interference figures are produced, consisting of dark or colored rings and crosses. It is impossible to enter here into the optical principles which condition these phenomena; but the difference between the circular figures produced by uniaxial crystals, and the elliptical appearances characteristic of biaxial crystals (Fig. 58), are easily apparent. With some sections which show only an indistinct bar instead of a clear figure, if the stage be rotated, the bar follows it and remains straight, in the case of uniaxial crystals, while with biaxial crystals it moves in the opposite direction and becomes curved. Quartz and gypsum will again serve for comparison, the former being uniaxial, the latter biaxial.

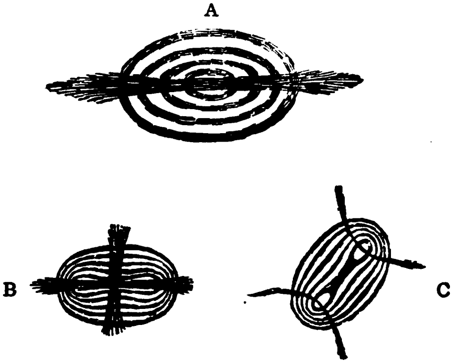

FIG. 58.—INTERFERENCE FIGURES. (After Luquer.)

Two more characters remain to be noted, absorption and pleochroism. Absorption is the power of absorbing all or part of the light which vibrates in certain planes, and pleochroism the similar property of removing the rays of certain colors in particular planes. If a rock section be examined with parallel rays of polarized light and the stage be rotated, minerals which exhibit absorption will show a change in intensity of light from the usual shade almost to black, while pleochroic minerals show a change in color, or at least in shade.

We have now data sufficient to identify any one of the six crystallographic systems, whose optical characters may be briefly summarized as follows: Isometric system: isotropic, exhibiting no interference figures in convergent light. Tetragonal system: anisotropic, uniaxial, extinction symmetrical, sections giving uniaxial interference figures, often with rectangular cleavage. Hexagonal system: anisotropic, uniaxial, extinction symmetrical, sections giving uniaxial interference figures, three- or six-sided, or show cleavage lines intersecting at angle of 60°. Orthorhombic system: anisotropic, biaxial, extinction symmetrical in all sections. Monoclinic system: anisotropic, biaxial, extinction symmetrical in one zone, while other sections show extinction angles. Triclinic system: anisotropic, biaxial, extinction angles in all sections. By application of these criteria, combined with observation of the more obvious optical characters, color, aggregation, etc., it is possible to identify any of the commoner minerals with ease and certainty.

4. **The Structure and Behavior of Alloys.**—Metallography, or the study of the structure of metals with the

microscope, owes its beginnings to Dr. Early of Sheffield and Professor Martens of Charlottenburg. Its aim is to study the minute structure of metals with the microscope, not, as in the case of minerals, by grinding off thin sections, but by examining polished surfaces by reflected light. Its importance is now so great in the testing of iron and steel as to demand some attention in the most elementary survey of the field of microscopy; and since it is with the various alloys that metallography is principally concerned, certain general characteristics of these bodies must first be briefly discussed.

Many alloys may, for practical purposes, be considered as solutions of one metal in another; the alloy of silver and copper is a good example of this type. If a molten alloy containing more than 72% of silver be gradually cooled, a point is reached at which the rate of cooling becomes retarded and silver begins to separate. The greater the amount of silver the higher is the temperature at which this process begins. After a time, when the temperature falls to 770° C., the still molten silver will be found to have fallen to just 72%; a second retardation in the cooling is now apparent accompanied by the solidification of the entire alloy. If more than 28% of copper be present, on the other hand, the first retardation is accomplished by a deposition of copper, which continues until the excess has all been solidified, which occurs at a temperature of 770° C. The remainder then solidifies entire. A molten alloy containing originally 72% of silver and 28% of copper shows only one point of retardation, remaining molten above 770° and becoming solid below that temperature. The relation of these various points is made more clear in Fig. 59, in which the outer limits of the oblique lines represent the fusing-points of pure silver and copper.

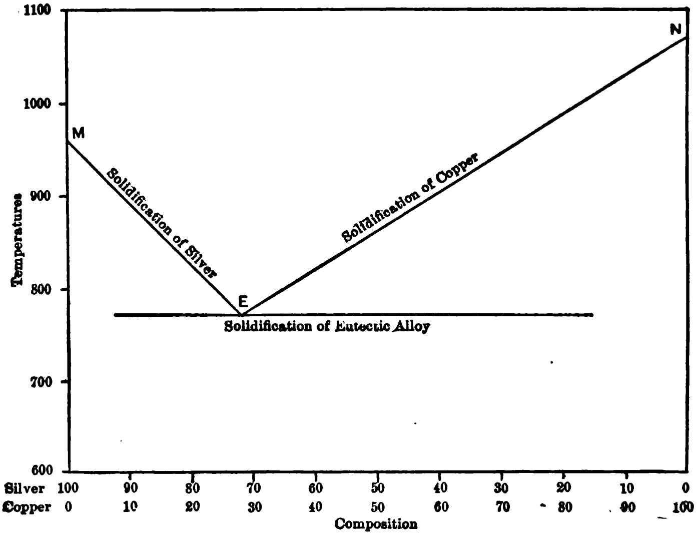

FIG. 59.—CURVE OF SOLIDIFICATION FOR ALLOY OF SILVER AND COPPER. (After Sauveur.)

The alloy of such composition as to show but one point of solidification, the lowest one possible for any mixture of the metals concerned, is known as the eutectic alloy. From any other mixture the metal which is in excess crystallizes out first until the proper proportions are reached for the formation of the eutectic. Similar phenomena are manifest in ordinary solutions, and the solution of such strength as to show a single minimum freezing-point is called the cryohydrate.

5. **Metallography of Steel.**—Steel is practically an alloy of ferric carbide and iron, and the phenomena attending its cooling from a high temperature are essentially the same as those we have just considered. The compound corresponding to the eutectic alloy contains 12% Fe_3C, or 0.8% of pure carbon. This substance is known as pearlyte, and steel of the right composition on gradual cooling from a fused condition becomes entirely converted into it at a temperature of 670° C. If more carbon be present, ferric carbide is separated before this temperature be reached; if iron, that metal is segregated. The carbide is known to microscopists as cementite, the pure iron as ferrite. Very low carbon steels are made up of ferrite, with here and there particles of pearlyte. The proportion of pearlyte increases with an increase of carbon up to 0.8%, after that point being mixed with cementite.

If steel be suddenly cooled from a high temperature, above that at which segregation begins, a new compound is formed known as martensite, and to this substance is due the intense hardness of steel thus quenched. Below the point at which ferrite or cementite begins to separate, sudden cooling produces a mixture of these bodies with martensite.

If a smooth surface of steel be examined under the microscope the presence and the general proportions of these various compounds may be readily made out. The specimen, of some convenient size, say from half an inch to an inch square and half an inch thick, is first very carefully polished upon one face with emery and with rouge. The final polishings are carried out by rubbing the metal on a layer of parchment stretched over smooth wood and covered with moist rouge to which a little ammonia-water has been added. In this latter process the softer parts are somewhat eroded and the harder structures stand out in low relief. The specimen when finally prepared is examined with an ordinary microscope provided only with some apparatus for illuminating with reflected light. This may consist merely of a condenser or reflector which throws light obliquely on the stage, or better, of a plane reflector or right-angled prism fitting into the tube of the microscope above the objective. This is perforated at its center to allow passage of rays upward from the object, while the remainder of its surface reflects light from without vertically downward on the specimen.

When examined in this fashion with the high objective, pearlyte is seen to be made up of very minute crystals of two substances arranged alternately; this is the characteristic structure of all eutectic alloys, and indicates that in spite of their constancy of composition these bodies are merely peculiarly intimate mixtures of the two constituent substances. Cementite, if present, appears in large whitish masses; being harder than pearlyte, it stands out in relief. Ferrite, on the other hand, is the softest material in steel; and in alloys with more than 88% of free iron pearlyte areas appear standing out from a background of ferrite. Fig. 60 illustrates the structure of mixed pearlyte and cementite as seen under the microscope. If martensite be present it appears as a homogeneous substance made up of crystalline needles easily distinguished from any other constituents of steel.

The exact amount of carbon in steel must be determined by chemical methods; but the presence of the mineral forms we have discussed and the manner in which they are associated can only be ascertained under the microscope. Furthermore, the crystals present may be abnormally arranged, being perhaps not closely packed or so large as to produce incipient cleavage planes.

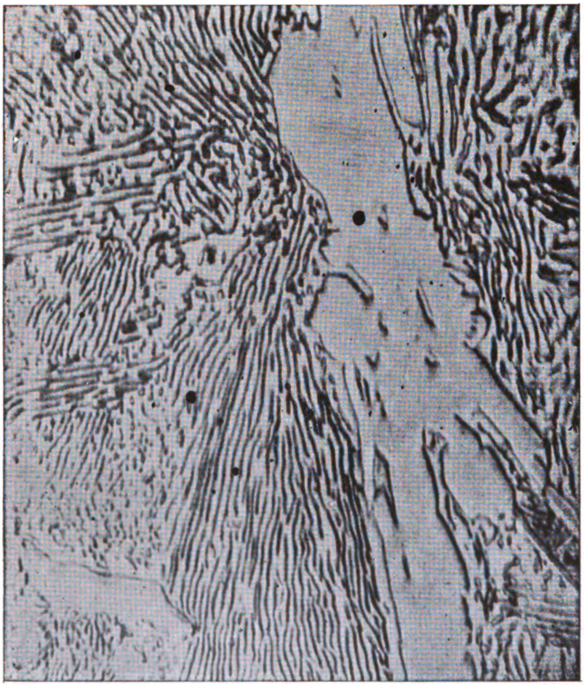

FIG. 60.—MICROSCOPIC APPEARANCE OF STEEL WITH 1.5% CARBON (PEARLYTE AND CEMENTITE). (After Sauveur.)

All these factors are of great importance in determining the hardness of steel; it is therefore natural that the microscope should have become indispensable in the laboratory of the iron and steel expert. Its practical application to the study of other alloys promises extensive development in the future.

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